Published in final edited form as:

Bioessays. 2018 May ; 40(5): e1700243. doi:10.1002/bies.201700243.

A new way to treat brain tumors: Targeting proteins coded by microcephaly genes?:

Brain tumors and microcephaly arise from opposing derangements regulating progenitor growth. Drivers of microcephaly could be attractive brain tumor targets

Patrick Y. Lang^{1,2} and Timothy R. Gershon^{2,3,4}

¹Department of Cell Biology and Physiology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599, USA

²Department of Neurology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599, USA

³Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina 27599, USA

⁴Neuroscience Center, University of North Carolina, Chapel Hill, North Carolina 27599, USA

Summary

New targets for brain tumor therapies may be identified by mutations that cause hereditary microcephaly. Brain growth depends on the repeated proliferation of stem and progenitor cells. Microcephaly syndromes result from mutations that specifically impair the ability of brain progenitor or stem cells to proliferate, by inducing either premature differentiation or apoptosis. Brain tumors that derive from brain progenitor or stem cells may share many of the specific requirements of their cells of origin. These tumors may therefore be susceptible to disruptions of the protein products of genes that are mutated in microcephaly. We highlight the potential for the products of microcephaly genes to be therapeutic targets in brain tumors by reviewing research on EG5, KIF14, ASPM, CDK6, and ATR. Treatments that disrupt these proteins may open new avenues for brain tumor therapy that have increased efficacy and decreased toxicity.

Graphical Abstract

Microcephaly and brain cancer exist on opposite ends of a spectrum defined by regulated proliferation of neural stem/progenitor cells. Several genes that are under-expressed in microcephaly are either over-expressed or critically important in brain cancers. We propose studying the products of these genes to develop new brain cancer treatments.

Corresponding author: Timothy R. Gershon, gershont@neurology.unc.edu.

Author contributions: P.Y.L. and T.R.G. conceived, wrote, and edited the manuscript.

Conflict of interest: The authors declare no potential conflicts of interest.

Competing interests

The authors declare no competing or financial interests.

Keywords

microcephaly; glioma; medulloblastoma; targeted therapy; mitosis

1. Introduction

During brain development, a small number of pluripotent stem cells generates a much larger number of brain cells through many rounds of cell division. This process involves the generation of neural progenitors, which are transit amplifying cells that are highly proliferative but less pluripotent^[1]. Strict control of neural stem and progenitor cell proliferation prevents both developmental malformations resulting from *hypo*-proliferation and tumor formation resulting from *hyper*-proliferation.

Microcephaly is a neurodevelopmental disorder in which the growth of the brain is disproportionately reduced, relative to the growth of the body. Diverse pathologic processes can cause microcephaly, ranging from genetic conditions to fetal trauma to maternal exposures. The common link between these processes is insufficient proliferation, specifically in neural stem or progenitor cells. Reduced proliferation may be caused by some combination of inappropriate cell death and loss of proliferative potential through premature differentiation or senescence^[2]. Heritable microcephaly syndromes result from mutations in genes that are specifically required by neural stem or progenitor cells to maintain their survival and proliferation. These hereditary conditions identify gene products that critically regulate processes necessary for the normal expansion neural stem and progenitor cell populations.

The cells of primary brain tumors descend from neural stem and progenitor cells and their proliferation may depend on the same genes that are required by the normal proliferative cells within their lineage. The most common primary, malignant brain tumors are glioblastoma in adults and medulloblastoma in children. The cell of origin for glioblastoma is still under investigation, but candidates include Nestin⁺ neural stem cells, multipotent progenitors of the subventricular zone, or lineage-restricted progenitors that give rise to astrocytes or oligodendrocytes^[3–5]. Medulloblastomas of specific subgroups arise from

either neural stem cells of the cerebellar ventricular zone or progenitor cells of the external granule layer of the cerebellum or rhombic lip [6]. In both glioblastoma and medulloblastoma, either a majority or a distinct population of tumor cells retain genetic and molecular characteristics of the stem/progenitor cell of origin [7]. Therefore, proteins that support growth and survival in neural stem/progenitor cells may retain their function in brain tumors. Familial microcephaly and congenital microcephalic disorders identify these proteins, which may be attractive targets for brain tumor treatment.

In this review, we focus on five genes that are each mutated in a different form of hereditary microcephaly and either over-expressed or uniquely important in a primary brain tumor: *KIF11*, *KIF14*, *ASPM*, *CDK6*, and *ATR*. The products of these genes all exert some form of control over mitotic entry or progression, underscoring the particular importance of mitosis for normal brain development and tumor growth. For each of the five candidates, preclinical studies have demonstrated efficacy in targeting the gene products for attenuating tumorigenesis in glioblastoma and/or medulloblastoma. These examples ultimately highlight the importance of studying normal brain development for understanding the pathogenesis and treatment of brain tumors.

2. *KIF11* is mutated in primary microcephaly and upregulated in glioma

KIF11 mutations were initially identified in patients with primary, autosomal dominant microcephaly [8]. Examination of ten families found diverse *KIF11* mutations that were associated with microcephaly, chorioretinopathy, and/or lymphedema. Heterozygous nonsense mutations and single- and di-nucleotide deletions were observed, with no clear association between genetic alteration and phenotype, including in later analyses encompassing more subjects [9]. All of the mutations were predicted to alter the *KIF11* protein product, EG5, which is a bipolar molecular motor protein of the kinesin family. *KIF11* expression begins early in mammalian development, at the blastula stage and EG5 is required for initial cleavage events in embryogenesis [10]. The autosomal dominant pattern of inheritance shows that heterozygous mutations are sufficient to disrupt EG5 function, and suggest that homozygous deletion may not be viable. Consistent with this conclusion, *Kif11*-null mouse embryos perish prior to gastrulation [11].

EG5 has been shown to interact with microtubules of the mitotic spindle during mitosis [12]. Chromatid separation in mitosis requires formation of a bipolar spindle apparatus with two centrosomes located on opposite sides of the nucleus (Fig. 1A). The hetero-tetrameric shape and plus-end-directed motor activity of EG5 allow it to crosslink antiparallel microtubules and push apart the duplicated centrosomes in prophase [13, 14]. Continued EG5 activity into prometaphase completes establishment of a bipolar mitotic spindle that is capable of pulling apart sister chromatids [15, 16]. Indeed, early studies of EG5 disruption using the small molecule inhibitor monastrol resulted in monoasters with radial spokes of microtubules surrounded by a band of duplicated, unsegregated chromosomes (Fig. 1A') [12, 17].

Formation of this aberrant mitotic spindles on EG5 disruption leads to activation of the spindle assembly checkpoint via MAD2 and subsequent mitotic arrest [12, 18]. Prolonged mitotic arrest triggers the cell death program through the intrinsic apoptotic pathway and

activation of Caspase-3 [19]. The exact mechanism, however, for how mitotic arrest actually leads to apoptosis is an area of active investigation. One theory proposes that prolonged mitotic arrest produces cellular stress, which induces activation of Caspases-9 and -7 and the caspase-activated DNase [20]. This nuclease damages the DNA of arrested cells, triggering full engagement of the p53-dependent apoptotic pathway.

Although it is still unclear how exactly *KIF11* mutation leads to microcephaly, it follows that based on the known mechanism of EG5 and the consequences of its inhibition in other cells, that reduced EG5 activity may increase apoptosis in mitotically active neural stem and progenitor cells. In the developing zebra fish nervous system, eg5 inhibition was found to attenuate the rate of proliferation and increase the rate of cell death in neural stem and progenitor cells [21]. Most likely through a similar mechanism, *KIF11* mutations that reduce rather than completely abrogate EG5 activity specifically impair brain growth in humans.

Importantly, while EG5 acts as an essential microtubule motor during mitosis, this function is *limited* to mitosis [12, 17]. That is, EG5 does not seem important in for the non-mitotic functions of microtubules, which include diverse cellular processes, among them axonal transport in the central nervous system [22]. Thus, EG5 inhibitors may disrupt the mitotic spindle without producing the neurotoxicity of microtubule-directed agents such as vinca alkaloids (vinblastine and vincristine) and taxanes (paclitaxel and docetaxel) [23, 24]. Overexpression of *KIF11* has been identified in bladder and pancreatic cancers and its expression was correlated with higher clinical grades and stages [25, 26]. A recent study showed that *KIF11* knockdown reduced proliferation and increased apoptosis in triple-negative breast cancer cells [27]. Increased *KIF11* expression in triple-negative breast cancer was associated with shortened disease free survival. A 2015 study found *KIF11* upregulation in human glioblastoma and showed that EG5 inhibition by the small molecule inhibitor ispinesib (SB-715992) reduced the proliferation of neural stem-like glioblastoma cells *in vitro* and in mouse xenograft models by preventing mitotic progression [28]. In this study, Ispinesib treatment increased the survival of mice with glioblastoma from 24 to 36 days.

KIF11/EG5 inhibitors have not yet been tested for primary brain cancers in clinical trials in the United States. However, several small molecule EG5 inhibitors have been tested in patients with various other cancers, including ispinesib, litronesib (LY-2523355), filanesib (ARRY-520), SB-743921, 4SC-205, AZD-4877, MK-0731, and ARQ-621 (Table 1). For all of these agents, patients experienced predictably minimal neurotoxicity – neutropenia and leukopenia were more common side-effects since mitotic inhibition targets all rapidly dividing cells [48]. Unfortunately, EG5 inhibition as monotherapy had limited benefit in the cancers in which it has been tested, due to the short half-life of many of the inhibitors, which prevented targeting relatively slower cycling neoplastic cells in those cancers [49]. ARRY-520 and 4SC-205 have shown longer half-life, but in general, EG5 inhibitors are being tested in combination with other chemotherapeutics [50]. There is currently only one active clinical trial for an EG5 inhibitor: ARRY-520 for multiple myeloma. Regardless of these findings in other cancers, EG5 inhibition could potentially still hold great promise even as monotherapy for glioblastoma since the cancer cells of glioblastoma are very highly proliferative, which may allow even short half-life inhibitors to exert a potent effect. Moreover, the recent reports on EG5 inhibition in preclinical models of glioblastoma [19, 28]

are encouraging and recommend further exploration of inhibiting EG5 for the treatment of high grade gliomas.

3. *KIF14* is mutated in primary microcephaly and upregulated in glioma and medulloblastoma

KIF14 is another member of the kinesin family and, like *KIF11*, acts as a mitotic microtubule motor protein. Microarray analysis in synchronized cells shows maximal *KIF14* expression during mitosis [51]. In addition, the minimal amount of *KIF14* in interphase is localized to the cytoplasm, whereas mitotic progression both increases *KIF14* expression and induces protein entry into the nucleus. siRNA silencing of *KIF14* prevents cytokinesis, leading to either immediate apoptosis or the formation of unstable, multinucleated cells that later undergo apoptosis.

The first suggestion that *KIF14* mutation could induce microcephaly came about incidentally during the breeding of genetically engineered mice to study neurite outgrowth [52]. Homozygous *KIF14* mutation arose spontaneously and mutant mice were found to have microcephaly and severely disrupted CNS myelination. The authors found that cerebral and cerebellar hypoplasia were a result of increased apoptosis and reduced proliferation during embryogenesis. Recently, *KIF14* mutation was identified as the driver of a form of primary, autosomal recessive microcephaly in four families [53]. These independent families were from Pakistan, Saudi Arabia, and Germany, and encompassed a total of ten individuals with microcephaly and homozygous and compound heterozygous *KIF14* mutations that resulted in aberrant splicing or truncated protein. Molecular analyses showed that in primary patient-derived fibroblasts, mutant *KIF14* failed to appear at the cytokinesis midbody. Absence of *KIF14* was associated with concurrent absence of the critical cytokinesis regulator CRK [54]. As a result, *KIF14*-mutated cells from these microcephalic patients tended to be either binucleated or apoptotic. Finally, two disparate inactivating biallelic mutations in *KIF14* were found in two non-viable human fetuses that had severe microcephaly, intrauterine growth restriction, renal dysplasia/agenesis, and other genitourinary abnormalities [55]. In contrast to mutations detected in living patients with microcephaly, these mutations were detected in post-mortem analysis of non-viable fetuses [55], and were thus selected for greater severity, rather than for viability. All of the *KIF14* phenotypes require homozygous or compound heterozygous *KIF14* mutations, demonstrating that unlike *KIF11*, a single unaffected copy of *KIF14* is sufficient to sustain neural development.

In cancer, *KIF14* has been found to be up-regulated in diverse types of tumors. Retinoblastoma was among the first tumors where *KIF14* overexpression was noted [56], and *KIF14* overexpression was found to accelerate retinoblastoma tumorigenesis in a murine model [57]. *KIF14* overexpression has also been found in both ovarian and chemo-resistant breast cancers, where *KIF14* inhibition leads to chemo-sensitization [58, 59]. Interestingly, one study found reduced levels of *KIF14* in lung adenocarcinomas, and levels of *KIF14* mRNA and protein were inversely correlated with overall survival and metastasis [60]. *In vitro* and *in vivo* experiments demonstrated that increased expression of *KIF14* inhibited lung adenocarcinoma growth.

In gliomas, *KIF14* was first found to be upregulated in 99/128 patient-derived samples, and increased levels of both *KIF14* mRNA and protein corresponded to worse pathological grade [61]. *KIF14* expression correlated with higher proliferative rate and mitotic index in gliomas. These characteristics were associated with poorer outcomes in glioma patients with *KIF14* overexpression. Results from a separate study also found increased *KIF14* expression in glioblastoma cell lines and 53 human astrocytoma samples, where again, higher levels of *KIF14* corresponded to worse pathological grade [62]. siRNA knockdown of *KIF14* in glioblastoma cell lines increased G₂/M arrest, cytokinesis failure leading to binucleated cells, and apoptosis. Furthermore, tumors in mice xenografted with cells from glioblastoma cell lines were smaller if treated with *KIF14* siRNA.

In a miRNA expression analysis by small RNA-Seq on 416 pediatric gliomas, miR-137 and miR-6500-3p were found to be significantly downregulated [63]. Reduced expression of these miRNA was associated with increased expression of *KIF14*. As in the prior studies, higher grade pediatric gliomas had greater levels of *KIF14*, and these patients had worse outcomes. *KIF14* knockdown in pediatric high-grade glioma cell lines led to reduced proliferation due to G₁ arrest, and the anti-proliferative effect was enhanced by concomitant temozolomide treatment.

Finally, *KIF14* expression was found to be significantly elevated in 7 medulloblastoma cell lines and 32/32 human medulloblastoma tumor samples [64]. Analysis on a separate group of 93 medulloblastoma samples demonstrated that, as in glioma, greater *KIF14* expression corresponded to shorter progression-free survival and overall survival. Interestingly, lower *KIF14* levels were seen in the WNT subgroup of medulloblastoma, which tends to have better outcomes, and higher *KIF14* levels were seen in the other medulloblastoma subgroups. The authors also found increased *KIF14* expression in tumor samples with gain of chromosome 1q, which is associated with non-WNT medulloblastoma. *In vitro*, *KIF14* siRNA knockdown in two medulloblastoma cell lines attenuated proliferation, impaired cell migration and invasion, and promoted apoptosis. Taken together, these findings should inspire further research on *KIF14* inhibition in animals with primary, spontaneous brain cancer. As of now, there are currently no *KIF14* inhibitors in clinical trials in the United States.

4. *ASPM* is mutated in primary microcephaly and upregulated in glioma and medulloblastoma

Like EG5, *ASPM* has a role in orienting and maintaining the mitotic spindle, and it is especially important in neural stem/progenitor cells [65, 66]. *ASPM* is a microtubule minus-end-associated protein that is found at the mitotic spindle poles from prophase through telophase [65, 67]. *In vitro* studies in non-neural human cells demonstrated that *ASPM* is required for cell division and that cytokinesis fails in its absence [67]. In *Drosophila* neuroblasts, the *ASPM* homolog *asp* is required for proper completion of mitosis and its disruption leads to mitotic arrest at metaphase [68]. In contrast, loss of *ASPM* in mammalian neural stem and progenitor cells does not prevent mitotic progression [69–71]. Rather, *ASPM* is thought to control symmetric divisions in epithelial stem cells of the neuroectoderm and

its acute inhibition by RNAi led to increased asymmetric divisions that depleted the stem cell population [69]. A conflicting report by the same group found no such changes to the proportion of symmetric versus asymmetric neuroepithelial divisions when *Aspm* was mutated. However, the authors suggested that this discrepancy could be due to acute, complete blockage of ASPM function in the ASPM-RNAi experiments versus persistent, partially preserved ASPM function in the experiments with *Aspm* mutation [70]. In the more lineage-committed cerebellar granule neuron progenitor (CGNP), ASPM mediates cell cleavage plane orientation [71]. Conditional *Aspm* deletion in CGNPs led to a relative increase in the proportion of divisions oriented transverse to the pial surface in the external granule layer of the early postnatal mouse cerebellum, consistent with inappropriately increased progenitor cell cycle exit and differentiation (Fig. 1B, B').

Homozygous mutations in *ASPM* are the most common cause of familial microcephaly [72, 73]. Nearly all of the identified 150+ identified mutations lead to ASPM truncation, primarily as a result of nonsense or frameshift mutations [74]. Although the mutations affect all tissues, for unclear reasons, ASPM is particularly important to neural stem/progenitor cells compared to other proliferating cells [65, 75]. Indeed, *Aspm*-null mice develop microcephaly and hypogonadism, but other tissues seem relatively unaffected [70, 76]. Similarly, individuals with homozygous *ASPM* mutations uniformly exhibit microcephaly and neurocognitive disorders, with varying degrees of other pathology such as behavioral problems, seizures, and rarely various organ malformations [74]. Research into the molecular function of ASPM indicates that microcephaly associated with *ASPM* mutation is due a combination of increased apoptosis and impaired self-renewal of neural stem and progenitor cells [69–71]. In brain progenitors, *Aspm* deletion provoked DNA damage and p53-dependent cell death [71], while increased cell death did not appear to occur in ASPM-inhibited neuroepithelial cells [70]. Altogether, these studies emphasize the disproportionate importance of ASPM in the developing brain compared to other tissues and demonstrate how ASPM disruption decreases proliferation and increases cell death in neural stem/progenitor populations.

In contrast to microcephaly, *ASPM* expression is increased in epithelial ovarian cancer and malignant gliomas. ASPM protein levels were correlated with epithelial ovarian cancer grade, as seen in cells isolated from human ovarian ascites samples [77]. The same authors validated these findings in a later study on primary patient tissue samples [78]. In an early study using 120 patient samples of glioblastoma, an unbiased screen identified *ASPM* as being significantly overexpressed [79]. A separate study using RT-PCR (reverse transcription polymerase chain reaction) and immunohistochemistry on tumor samples from 15 patients with low-grade (II) astrocytoma and 15 patients with glioblastoma showed that ASPM mRNA and protein were low in the astrocytomas but much higher in glioblastomas [80]. Similar results were obtained from 175 patient glioma samples, representing 8 grade II, 75 grade III, and 92 grade IV gliomas: ASPM mRNA and protein levels increased with tumor grade and were also higher in recurrent tumors [81]. siRNA knockdown of *ASPM* in glioblastoma cell lines reduced proliferation, increased cell death, and sensitized cells to DNA damaging agents like x-rays and cisplatin [79, 81, 82]. ASPM is also upregulated in medulloblastoma [83]. In a primary mouse model of medulloblastoma, conditional *Aspm* deletion was found to increase DNA damage in tumors and attenuate overall tumorigenesis

[71]. Further research *in vivo* is needed to illuminate the exact mechanism by which ASPM disruption reduces tumor formation or destroys tumors. Nonetheless, these studies highlight the shared dependence of neural stem/progenitor and brain tumor cells on ASPM. Despite these promising findings, there are currently no ASPM inhibitors in clinical trials in the United States.

5. CDK6 is mutated in primary microcephaly and upregulated in glioma and medulloblastoma

Data collected on eight generations of a large, consanguineous family in which 10 individuals had apparent primary, autosomal recessive microcephaly found recurrent mutations in *CDK6* [84]. CDK6 protein levels were not reduced in the microcephalic patients, however its function and behavior were altered. In control human fibroblasts, CDK6 was associated with centrosomes in mitosis (Fig. 1C) – this association was absent in *CDK6* mutant fibroblasts, which displayed abnormal mitotic spindle alignment and supernumerary centrosomes that persisted into interphase (Fig. 1C'). Mutant patient fibroblasts had abnormal cell polarity and reduced cell motility *in vitro*. While these observations have yet to be confirmed directly in neural stem or progenitor cells, the authors detected CDK6 by immunofluorescence in neuroepithelial and neural progenitor cells of the embryonic mouse brain. They hypothesized that microcephaly was a consequence of increased neural stem/progenitor apoptosis and decreased cell proliferation due to reduced RB phosphorylation–phenomena that were seen in the mutant fibroblasts.

The most well-studied role for CDK6 is in control of the cell cycle. CDK6 interacts with cyclin D in G₁-phase to phosphorylate RB [85]. This phosphorylation allows cell cycle progression from G₁- to S-phase [86, 87]. Cyclin D binding is required for CDK6 activity, and the levels of this cyclin are controlled by growth factors and mitogens [88]. In turn, CDK6 levels are thought to be regulated by p21 (CIP1), p27 (KIP1), and p57 (KIP2), which may control CDK6 assembly and nuclear translocation [89–91]. Besides cyclin D, CDK6 activity is also regulated by its inhibitors p15 (INK4B), p16 (INK4A), p18 (INK4C), and p19 (INK4D) [92].

Tight control of CDK6 cell cycle activity is imperative as it is expressed at some point in almost all tissues. Nevertheless, CDK6 has a homolog in mammals, CDK4, that is able to carry out many of the same functions [93, 94]. Indeed, *Cdk6*-null mice are viable and mostly develop normally, but suffer from anemia and defective T-cell maturation [95, 96]. *Cdk4*-null mice are also viable and instead experience pancreatic and pituitary hypoplasia [97, 98]. In contrast, loss of both *Cdk4* and *Cdk6* results in embryonic lethality, underscoring redundant roles for CDK4 and CDK6 [96]. The distinct phenotypes of the two null mice, however, suggest some non-overlapping functions for the kinases. Apart from a role in hematopoiesis, CDK6 may regulate the apoptotic program [99]. Interestingly, cultured mouse embryonic fibroblasts overexpressing *Cdk6* upregulated *Tp53* and subsequent exposure to UV irradiation caused rapid, widespread, BAX-mediated apoptosis.

In the CNS, *Cdk6* expression is high in cortical progenitor and striatal stem cells and decreases once the cells cease proliferating and differentiate [100]. *Cdk6* disruption

prolonged G₁-phase in these cells and inhibited entry into S-phase. G₁-phase lengthening has been shown in mouse neuroepithelial cells to induce premature differentiation, explaining the decreased proliferation associated with *Cdk6* disruption [101]. Juvenile mice lacking *Cdk6* similarly showed precursor cells in the subventricular zone and subgranular zone of the dentate gyrus with prolonged G₁-phase, premature cell cycle exit, and attenuated proliferation [102]. Another role for CDK6 in the CNS is suggested by an experiment where astrocytes explanted from neonatal mice that were induced to overexpress *Cdk6* appeared more bi-polar and fibroblast-like than normal, multi-polar astrocytes [103]. Unlike in fibroblasts, increased expression of *Cdk6* in glia and neurons was not associated with apoptotic priming.

CDK6 upregulation is found in many cancers, including lymphoma, leukemia, bladder cancer, pancreatic cancer, and prostate cancer, as has been reviewed elsewhere [104]. In the brain, increased *CDK6* expression can be found in malignant glioma and medulloblastoma [105–107]. *CDK6* expression in gliomas increases with higher tumor grade and is correlated with a worse prognosis [108, 109]. siRNA knockdown of *CDK6* in cultured glioblastoma cells reduced proliferation and increased apoptosis following treatment with temozolomide [109]. Two miRNAs, miRNA-495 and miRNA-340, have been identified that suppress CDK6 activity [108, 110]. They are both downregulated in human glioblastoma and their expression was found to arrest tumor cells in G₁, leading to less proliferation. SUMO1 has also recently been identified to interact with CDK6 in glioblastoma, preventing CDK6 degradation, which permits tumor cell hyper-proliferation [111]. As in glioblastoma, CDK6 inhibition in medulloblastoma cell lines was found to induce G₁ arrest due to reduced RB phosphorylation, which attenuates cell proliferation [112]. Moreover, CDK6 inhibition significantly limited the survival of medulloblastoma cells exposed to radiation. The role of CDK6 in mitotic progression, as suggested by microcephaly, has yet to be investigated in these cancers.

A multitude of CDK6 inhibitors, though not all specific for CDK6, have been tested in clinical trials over the past twenty years for a variety of cancers [113]. Few, however, have been tried in primary brain cancers (Table 2). The CDK4/6 inhibitor palbociclib (PD-0332991) has the most ongoing clinical trials for mostly breast cancers, but also several for primary or metastatic brain tumors. Most promisingly, palbociclib reduced tumor growth in a patient-derived xenograft model of medulloblastoma, extending animal survival [118]. Ribociclib (LEE-011), another CDK4/6 inhibitor, has active Phase I/II clinical trials for gliomas. Finally, abemaciclib (LY-2835219), also a CDK4/6 inhibitor, has one active Phase I trial for children with diffuse intrinsic pontine glioma. Early results with these agents as monotherapy in non-brain cancers have shown good clinical outcomes, neutropenia and some gastrointestinal toxicity being the most common limiting side-effects. However, anti-tumor effects have generally been modest and not all cancers are responsive. Further research will reveal the feasibility of targeting CDK6 in humans to treat primary brain tumors.

6. ATR is mutated in Seckel syndrome and plays an important role in medulloblastoma tumorigenesis

ATR is a serine/threonine kinase that activates substrates involved in DNA repair, cell cycle arrest, and apoptosis (Fig. 1D) [119]. Specifically, ATR responds to the presence of single-stranded DNA, which forms as a normal part of DNA replication and pathologically as a result of DNA damage [120]. *In vitro* studies demonstrate that in undamaged dividing cells, ATR checks genomic integrity at S-phase and slows cell cycle progression to prevent replication stress from causing DNA damage [121]. Conversely, single-stranded DNA resultant from DNA damage can cause ATR activation in all phases of the cell cycle [122–124]. The presence of DNA damage prompts ATR to act in two possible ways: cell cycle arrest and DNA repair if the damage is minimal, or apoptosis if the damage is extensive [125]. Although ATR can promote apoptosis, loss of ATR is actually associated with increased cell death due to the greater role of ATR in mitigating DNA damage and preventing cell death [126]. There is some debate as to how cell death proceeds from ATR deficiency. Cleaved Caspase-3 activation in *Atr*-deleted cells indicates that cell death occurs via apoptosis [127, 128]. In contrast to typical apoptosis after genotoxic stress, however, ATR-deficient cell death has been suggested to be p53-independent [127, 129], which is unexpected, given the pivotal role of p53 in bridging DNA damage with apoptosis [130].

The role of ATR in proliferating cells is especially important due to its ability to relieve physiologic replication stress, which would otherwise cause DNA damage [131]. Complete loss of ATR is embryonic lethal in mice, as widespread chromosome fragmentation precipitates caspase-mediated apoptosis [132]. In the brain, ATR is essential to cerebellar growth, where proliferation of CGNPs continues longer than almost all other neural cell types [133]. Conditional deletion of *Atr* in the embryonic mouse CNS was found to almost exclusively affect two areas of the developing brain – the ganglionic eminence, which produces cortical, striatal, and olfactory cells, and the nascent cerebellum [129]. Neural stem cells of the embryonic brain required ATR for normal proliferation and expansion, and its loss resulted in microcephaly, with especially prominent cerebellar hypoplasia, and early postnatal lethality. In the absence of ATR, neural stem cell populations of the embryonic ganglionic eminence and nascent cerebellum experienced extensive DNA damage that led to either apoptosis or impaired proliferation, respectively. Interestingly, inactivation of p53 neither significantly rescued nor exacerbated the ATR phenotype, despite partial attenuation of apoptosis in the forebrain, leading the authors to conclude that neuropathology from ATR loss is not p53-dependent.

The function of ATR appears to be slightly different in more lineage-committed neural progenitors at later stages of development. Our group has found that *Atr* deletion in CGNPs causes chromosomal damage and widespread apoptosis but no defect in proliferation at the early postnatal stage, when CGNP proliferation is maximal [134]. Cerebellar hypoplasia was partially rescued by co-deletion of either *Bax* and *Bak* or *p53*. However, in the *Atr/Bax/Bak*-deleted mice, pathology persisted due to continued activation of p53 by ATM in CGNPs, leading to p21-mediated cell cycle arrest and premature cell cycle exit. CGNPs in *Atr/p53*-deleted mice experienced caspase-independent cell death that likely induced cerebellar

hypoplasia. Furthermore, ATR loss was found to increase the fraction of CGNPs in M-phase, suggesting an important role for ATR in mediating mitotic entry in CGNPs. This checkpoint role is supported by the finding that *Atr* deletion significantly increased the fraction of CGNPs with a spectrum of chromosomal abnormalities (Fig. 1D'). Finally, sequencing and pathway analyses demonstrated the critical importance of p53 in mediating the response to ATR loss in CGNPs.

While ATR may have slightly different functions in neural stem cells compared to neural progenitors, the impact of its loss in these cell types clearly demonstrates its importance in brain development. This importance is highlighted by the congenital disorder Seckel syndrome, which is caused by hypomorphic mutations in *ATR* and characterized by intrauterine growth restriction, cryptorchidism, distinctive facies, pancytopenia, and microcephaly [135, 136]. Research into the pathogenesis of Seckel syndrome, and especially the effect of *ATR* deletion on neural stem/progenitor cells, as discussed above, has implications for developing novel brain cancer therapeutics. Apart from Seckel syndrome, *ATR* mutation has only been associated with one other condition in man, per a single report [137]. The authors found in 24 individuals a heterozygous mutation in *ATR* that attenuated ATR activation of p53, which led to a phenotype consisting of oropharyngeal cancer, skin telangiectasias, and abnormal hair, teeth, and nails.

The unique requirement of cerebellar progenitors for ATR [129] suggests that ATR may be an effective therapeutic target for the cerebellar cancer medulloblastoma [138]. Activating mutations in the SHH signaling pathway are responsible for 25% of human medulloblastoma [139], and CGNPs are thought to be the cells of origin for this molecular subset [6]. Indeed, genetically engineered mice with neuronal hyper-activation of the SHH pathway reproducibly develop cerebellar tumors that resemble human medulloblastoma [140]. As with other types of cancer cells, medulloblastoma cells suffer from genetic instability owing to rapid rounds of proliferation [141]. adiation and chemotherapy, the current standard of care for medulloblastoma, take advantage of this genomic instability by further damaging the DNA of neoplastic cells – enough to push them towards cell death [142, 143]. However, radiation can be extremely toxic to the normal brain, and chemotherapy damages healthy, endogenous dividing cells such as found in the GI tract and bone marrow [144]. These effects can be particularly devastating in young patients.

We have found that *Atr* deletion in two different mouse models of medulloblastoma either significantly reduced tumorigenesis or completely abrogated tumor formation [145]. Our group is currently testing pharmacological ATR inhibition in mice with established medulloblastoma using a novel formulation in which the small molecule ATR inhibitor VE-822 is packaged in polymeric micelle nanoparticles (pVE-822). Preliminary results show that pVE-822 delivered by intraperitoneal injection effectively crosses the blood-brain barrier and induces DNA damage and apoptosis in brain progenitors of neonatal wild-type mice. In older, medulloblastoma-bearing mice, pVE-822 treatment increased tumor DNA damage and reduced tumor size. Ongoing research aims to determine whether pVE-822 produces a survival benefit in mice with medulloblastoma and how pVE-822 may be combined with other therapies to improve treatment outcomes. At this time, VE-822, also known as VX-970 is in Phase I clinical trials with radiation therapy for secondary brain

cancer (metastases from non-small cell lung cancer). VX-970 is also being actively tested in Phase I and II trials for other non-brain malignancies (Table 3). In addition, the ATR inhibitor AZD-6738 is currently in Phase I trials for a variety of liquid and solid tumors. Similarly, BAY1895344 is in Phase I trials for advanced solid tumors and lymphomas. Thus far, increased ATR activity has only been described in male breast cancer [147].

A particular benefit of ATR inhibition for the treatment of brain cancers is that in the adult brain, ATR seems to be dispensable for the survival and function of post-mitotic neurons [145, 148, 149]. It has been shown in mice, however, that ATR is still required for normal proliferation of remaining neural stem cell populations in the adult mouse brain, such as in the hippocampus [150]. However, our findings in juvenile medulloblastoma-bearing mice suggest fairly minimal CNS toxicity. In gliomas, although *ATR* upregulation has yet to be reported, the deleterious effect of ATR disruption in the neural stem cells from which gliomas may derive supports investigating ATR inhibition in these tumors. Here especially, the apparent CNS tolerability for ATR disruption suggests the possibility of an improved therapeutic window for ATR inhibition over traditional chemotherapy.

7. Targeted disruption of microcephaly gene products, combined with standard therapies

A concern remains that inhibitors of mitotic gate-keepers may not synergize well with current treatments involving radiation and cytotoxic chemotherapy. The basis for this concern is that blocking mitosis would be expected to halt cell cycle progression and current therapies for brain tumors target cycling cells [151, 152]. However, the anti-mitotic agent vincristine has long been paired with alkylating agents and cisplatin in medulloblastoma therapy [153]. Moreover, disruption of *ASPM* and *ATR* does not block the cell cycle progression of neural stem/progenitor cells [69–71, 145]. The potential for combining the targeted disruption of microcephaly proteins with conventional radiation and chemotherapy is unknown, and must be investigated before conclusions can be drawn. An obvious advantage of targeted therapy would be reduced toxicity, such as seen in preclinical studies with *EG5* inhibition in glioblastoma and *ATR* inhibition in medulloblastoma.

8. Conclusions

We propose that the proteins encoded by genes that are mutated in microcephaly are critical for growth within the nervous system and may thus be important targets for brain tumor treatment. Of the five microcephaly associated proteins we discuss, three – *EG5*, *ASPM*, and *CDK6* – play a role in orienting the mitotic spindle in neural stem and progenitor cells [12, 65, 66, 84, 154]. Our group has shown that *ATR* similarly has an important function related to neural stem/progenitor cell mitosis, in that *ATR* controls mitotic entry in CGNPs [145]. *KIF14*, is also upregulated in mitosis and controls cytokinesis [51]. That multiple genes mutated in microcephalic disorders code for proteins involved with mitosis is not necessarily surprising since primary microcephaly has been described as essentially a mitotic disorder [155]. Rather, these examples emphasize the similar molecular dependencies of neural stem/progenitor cells and brain cancer cells and suggest that the disproportionate importance of mitosis in neural stem/progenitor cells should provide incentive to study mitotic inhibition

more intensively as a potential targeted treatment for brain cancers. While prior clinical trials with anti-mitotic agents like Aurora kinase inhibitors and polo-like kinase inhibitors have shown limited success, these studies were almost entirely in non-brain tumors. The limited efficacy of these inhibitors in humans was thought to be due in part to the longer doubling time of tumors in humans (average 114–391 days) compared to in animals (average 3.4–5.7 days) ^[156]. However, primary brain tumors in humans have much shorter doubling times (average 25–50 days) ^[157,158]. This difference in doubling time is likely just one of many reasons that disrupting microcephaly-associated proteins may be more effective in brain tumors than in other tumor types. We propose that inhibiting targets revealed by microcephalic disorders to be particularly required for growth in the brain will bring new efficacy and reduced toxicity to brain tumor treatment.

Acknowledgments

Financial support: P.Y.L. is supported in part by F30CA192832 from the National Cancer Institute. T.R.G. is supported in part by R01NS088219 from the National Institute of Neurologic Disorder and Stroke and by the Alex's Lemonade Stand Foundation.

Funding

This work was supported by the National Institute of Neurological Disorders and Stroke (R01NS088219 to T.R.G.) and the National Cancer Institute (F30CA192832 to P.Y.L.).

List of abbreviations

ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
CGNP	cerebellar granule neuron progenitor
CLL	chronic lymphocytic leukemia
CML	chronic myeloid leukemia
CNS	central nervous system
ECM	extracellular matrix
MDS	myelodysplastic syndrome
miRNA	micro-RNA
MM	multiple myeloma
NHL	non-Hodgkin's lymphoma
NSCLC	non-small cell lung cancer
PCL	plasma cell leukemia
PLL	prolymphocytic leukemia
RNAi	RNA interference

SCLC	small cell lung cancer
siRNA	small interfering RNA
XRT	x-ray therapy

References

1. Noctor SC, Martinez-Cerdeno V, Kriegstein AR. Arch Neurol. 2007; 64:639. [PubMed: 17502462]
2. Jamuar SS, Walsh CA. Pediatr Clin North Am. 2015; 62:571. [PubMed: 26022163]
3. Alcantara Llaguno S, Chen J, Kwon CH, Jackson EL, Li Y, Burns DK, Alvarez-Buylla A, Parada LF. Cancer Cell. 2009; 15:45. [PubMed: 19111880]
4. Holland EC, Celestino J, Dai C, Schaefer L, Sawaya RE, Fuller GN. Nat Genet. 2000; 25:55. [PubMed: 10802656]
5. Lindberg N, Kastemar M, Olofsson T, Smits A, Uhrbom L. Oncogene. 2009; 28:2266. [PubMed: 19421151]
6. Gibson P, Tong Y, Robinson G, Thompson MC, Currle DS, Eden C, Kranenburg TA, Hogg T, Poppleton H, Martin J, Finkelstein D, Pounds S, Weiss A, Patay Z, Scoggins M, Ogg R, Pei Y, Yang ZJ, Brun S, Lee Y, Zindy F, Lindsey JC, Taketo MM, Boop FA, Sanford RA, Gajjar A, Clifford SC, Roussel MF, McKinnon PJ, Gutmann DH, Ellison DW, Wechsler-Reya R, Gilbertson RJ. Nature. 2010; 468:1095. [PubMed: 21150899]
7. Dirks PB. J Clin Oncol. 2008; 26:2916. [PubMed: 18539973]
8. Ostergaard P, Simpson MA, Mendola A, Vasudevan P, Connell FC, van Impel A, Moore AT, Loeys BL, Ghalamkarpour A, Onoufriadis A, Martinez-Corral I, Devery S, Leroy JG, van Laer L, Singer A, Bialer MG, McEntagart M, Quarrell O, Brice G, Trembath RC, Schulte-Merker S, Makinen T, Vikkula M, Mortimer PS, Mansour S, Jeffery S. Am J Hum Genet. 2012; 90:356. [PubMed: 22284827]
9. Jones GE, Ostergaard P, Moore AT, Connell FC, Williams D, Quarrell O, Brady AF, Spier I, Hazan F, Moldovan O, Wiczorek D, Mikat B, Petit F, Coubes C, Saul RA, Brice G, Gordon K, Jeffery S, Mortimer PS, Vasudevan PC, Mansour S. Eur J Hum Genet. 2014; 22:881. [PubMed: 24281367]
10. Castillo A, Justice MJ. Biochem Biophys Res Commun. 2007; 357:694. [PubMed: 17449012]
11. Chauviere M, Kress C, Kress M. Biochem Biophys Res Commun. 2008; 372:513. [PubMed: 18474226]
12. Kapoor TM, Mayer TU, Coughlin ML, Mitchison TJ. J Cell Biol. 2000; 150:975. [PubMed: 10973989]
13. Kashina AS, Rogers GC, Scholey JM. Biochim Biophys Acta. 1997; 1357:257. [PubMed: 9268050]
14. Sawin KE, LeGuellec K, Philippe M, Mitchison TJ. Nature. 1992; 359:540. [PubMed: 1406972]
15. Blangy A, Lane HA, d'Herin P, Harper M, Kress M, Nigg EA. Cell. 1995; 83:1159. [PubMed: 8548803]
16. Kashina AS, Baskin RJ, Cole DG, Wedaman KP, Saxton WM, Scholey JM. Nature. 1996; 379:270. [PubMed: 8538794]
17. Mayer TU, Kapoor TM, Haggarty SJ, King RW, Schreiber SL, Mitchison TJ. Science. 1999; 286:971. [PubMed: 10542155]
18. Chin GM, Herbst R. Mol Cancer Ther. 2006; 5:2580. [PubMed: 17041103]
19. Valensin S, Ghiron C, Lamanna C, Kremer A, Rossi M, Ferruzzi P, Nievo M, Bakker A. BMC Cancer. 2009; 9:196. [PubMed: 19545421]
20. Orth JD, Loewer A, Lahav G, Mitchison TJ. Mol Biol Cell. 2012; 23:567. [PubMed: 22171325]
21. Johnson K, Moriarty C, Tania N, Ortman A, DiPietrantonio K, Edens B, Eisenman J, Ok D, Krikorian S, Barragan J, Gole C, Barresi MJ. Dev Biol. 2014; 387:73. [PubMed: 24370453]
22. Conde C, Caceres A. Nat Rev Neurosci. 2009; 10:319. [PubMed: 19377501]
23. Grisold W, Cavaletti G, Windebank AJ. Neuro Oncol. 2012; 14(Suppl 4):iv45. [PubMed: 23095830]

24. Marcus AI, Peters U, Thomas SL, Garrett S, Zelnak A, Kapoor TM, Giannakakou P. *J Biol Chem*. 2005; 280:11569. [PubMed: 15653676]
25. Ding S, Xing N, Lu J, Zhang H, Nishizawa K, Liu S, Yuan X, Qin Y, Liu Y, Ogawa O, Nishiyama H. *Int J Urol*. 2011; 18:432. [PubMed: 21449971]
26. Liu M, Wang X, Yang Y, Li D, Ren H, Zhu Q, Chen Q, Han S, Hao J, Zhou J. *J Pathol*. 2010; 221:221. [PubMed: 20455257]
27. Jiang M, Zhuang H, Xia R, Gan L, Wu Y, Ma J, Sun Y, Zhuang Z. *Oncotarget*. 2017; 8:92106. [PubMed: 29190901]
28. Venere M, Horbinski C, Crish JF, Jin X, Vasanji A, Major J, Burrows AC, Chang C, Prokop J, Wu Q, Sims PA, Canoll P, Summers MK, Rosenfeld SS, Rich JN. *Sci Transl Med*. 2015; 7:304ra143.
29. Beer TM, Goldman B, Synold TW, Ryan CW, Vasist LS, Van Veldhuizen PJ Jr, Dakhil SR, Lara PN Jr, Drelichman A, Hussain MH, Crawford ED. *Clin Genitourin Cancer*. 2008; 6:103. [PubMed: 18824433]
30. Lee CW, Belanger K, Rao SC, Petrella TM, Tozer RG, Wood L, Savage KJ, Eisenhauer EA, Synold TW, Wainman N, Seymour L. *Invest New Drugs*. 2008; 26:249. [PubMed: 17962907]
31. Knox JJ, Gill S, Synold TW, Biagi JJ, Major P, Feld R, Cripps C, Wainman N, Eisenhauer E, Seymour L. *Invest New Drugs*. 2008; 26:265. [PubMed: 18196204]
32. Shahin MS, Braly P, Rose P, Malpass T, Bailey H, Alvarez RD, Hodge J, Bowen C, Buller R. *J Clin Oncol*. 2007; 25:5562. [PubMed: 18065728]
33. Blagden SP, Molife LR, Seebaran A, Payne M, Reid AH, Protheroe AS, Vasist LS, Williams DD, Bowen C, Kathman SJ, Hodge JP, Dar MM, de Bono JS, Middleton MR. *Br J Cancer*. 2008; 98:894. [PubMed: 18319713]
34. Lee RT, Beekman KE, Hussain M, Davis NB, Clark JI, Thomas SP, Nichols KF, Stadler WM. *Clin Genitourin Cancer*. 2008; 6:21. [PubMed: 18501078]
35. Gomez HL, Philco M, Pimentel P, Kiyan M, Monsalvo ML, Conlan MG, Saikali KG, Chen MM, Seroogy JJ, Wolff AA, Escandon RD. *Anticancer Drugs*. 2012; 23:335. [PubMed: 22123335]
36. Holen KD, Belani CP, Wilding G, Ramalingam S, Volkman JL, Ramanathan RK, Vasist LS, Bowen CJ, Hodge JP, Dar MM, Ho PT. *Cancer Chemother Pharmacol*. 2011; 67:447. [PubMed: 20461380]
37. O'Connor OA, Gerecitano J, Van Deventer H, Afanasyev B, Hainsworth J, Chen M, Saikali K, Seroogy J, Escandon R, Wolff A, Conlan MG. *Blood*. 2009; 114:1673.
38. Infante JR, Patnaik A, Verschraegen CF, Olszanski AJ, Shaheen M, Burris HA, Tolcher AW, Papadopoulos KP, Beeram M, Hynes SM, Leohr J, Lin AB, Li LQ, McGlothlin A, Farrington DL, Westin EH, Cohen RB. *Cancer Chemother Pharmacol*. 2017; 79:315. [PubMed: 28097385]
39. Wakui H, Yamamoto N, Kitazono S, Mizugaki H, Nakamichi S, Fujiwara Y, Nokihara H, Yamada Y, Suzuki K, Kanda H, Akinaga S, Tamura T. *Cancer Chemother Pharmacol*. 2014; 74:15. [PubMed: 24752449]
40. LoRusso PM, Goncalves PH, Casetta L, Carter JA, Litwiler K, Roseberry D, Rush S, Schreiber J, Simmons HM, Ptaszynski M, Sausville EA. *Invest New Drugs*. 2015; 33:440. [PubMed: 25684345]
41. Shah JJ, Kaufman JL, Zonder JA, Cohen AD, Bensinger WI, Hilder BW, Rush SA, Walker DH, Tunquist BJ, Litwiler KS, Ptaszynski M, Orlowski RZ, Lonial S. *Cancer*. 2017; 123:4617. [PubMed: 28817190]
42. Mross KB, Scharr D, Richly H, Frost A, Bauer S, Krauss B, Krauss R, Mais A, Hauns B, Hentsch B, Baumgartner R, Scheulen ME. *J Clin Oncol*. 2014; 32:2564.
43. Gerecitano JF, Stephenson JJ, Lewis NL, Osmukhina A, Li J, Wu K, You Z, Huszar D, Skolnik JM, Schwartz GK. *Invest New Drugs*. 2013; 31:355. [PubMed: 22615058]
44. Kantarjian HM, Padmanabhan S, Stock W, Tallman MS, Curt GA, Li J, Osmukhina A, Wu K, Huszar D, Borthukar G, Faderl S, Garcia-Manero G, Kadia T, Sankhala K, Odenike O, Altman JK, Minden M. *Invest New Drugs*. 2012; 30:1107. [PubMed: 21494838]
45. Jones R, Vuky J, Elliott T, Mead G, Arranz JA, Chester J, Chowdhury S, Dudek AZ, Muller-Mattheis V, Grimm MO, Gschwend JE, Wulfing C, Albers P, Li J, Osmukhina A, Skolnik J, Hudes G. *Invest New Drugs*. 2013; 31:1001. [PubMed: 23329066]

46. Holen K, DiPaola R, Liu G, Tan AR, Wilding G, Hsu K, Agrawal N, Chen C, Xue L, Rosenberg E, Stein M. *Invest New Drugs*. 2012; 30:1088. [PubMed: 21424701]
47. Chen LC, Rosen LS, Iyengar T, Goldman JW, Savage R, Kazakin J, Chan TCK, Schwartz BE, Abbadessa G, Von Hoff DD. *J Clin Oncol*. 2011; 29:3076.
48. Knight SD, Parrish CA. *Curr Top Med Chem*. 2008; 8:888. [PubMed: 18673173]
49. Huszar D, Theoclitou ME, Skolnik J, Herbst R. *Cancer Metastasis Rev*. 2009; 28:197. [PubMed: 19156502]
50. Khoury HJ, Garcia-Manero G, Borthakur G, Kadia T, Foudray MC, Arellano M, Langston A, Bethelmie-Bryan B, Rush S, Litwiler K, Karan S, Simmons H, Marcus AI, Ptaszynski M, Kantarjian H. *Cancer*. 2012; 118:3556. [PubMed: 22139909]
51. Carleton M, Mao M, Biery M, Warren P, Kim S, Buser C, Marshall CG, Fernandes C, Annis J, Linsley PS. *Mol Cell Biol*. 2006; 26:3853. [PubMed: 16648480]
52. Fujikura K, Setsu T, Tanigaki K, Abe T, Kiyonari H, Terashima T, Sakisaka T. *PLoS One*. 2013; 8:e53490. [PubMed: 23308235]
53. Moawia A, Shaheen R, Rasool S, Waseem SS, Ewida N, Budde B, Kawalia A, Motameny S, Khan K, Fatima A, Jameel M, Ullah F, Akram T, Ali Z, Abdullah U, Irshad S, Hohne W, Noegel AA, Al-Owain M, Hortnagel K, Stobe P, Baig SM, Nurnberg P, Alkuraya FS, Hahn A, Hussain MS. *Ann Neurol*. 2017; 82:562. [PubMed: 28892560]
54. Bassi ZI, Audusseau M, Riparbelli MG, Callaini G, D'Avino PP. *Proc Natl Acad Sci U S A*. 2013; 110:9782. [PubMed: 23716662]
55. Filges I, Nosova E, Bruder E, Tercanli S, Townsend K, Gibson WT, Rothlisberger B, Heinemann K, Hall JG, Gregory-Evans CY, Wasserman WW, Miny P, Friedman JM. *Clin Genet*. 2014; 86:220. [PubMed: 24128419]
56. Corson TW, Huang A, Tsao MS, Gallie BL. *Oncogene*. 2005; 24:4741. [PubMed: 15897902]
57. O'Hare M, Shadmeh M, Sulaiman RS, Sishtla K, Sakisaka T, Corson TW. *Int J Cancer*. 2016; 139:1752. [PubMed: 27270502]
58. Singel SM, Cornelius C, Zaganjor E, Batten K, Sarode VR, Buckley DL, Peng Y, John GB, Li HC, Sadeghi N, Wright WE, Lum L, Corson TW, Shay JW. *Neoplasia*. 2014; 16:247. [PubMed: 24784001]
59. Theriault BL, Basavarajappa HD, Lim H, Pajovic S, Gallie BL, Corson TW. *PLoS One*. 2014; 9:e91540. [PubMed: 24626475]
60. Hung PF, Hong TM, Hsu YC, Chen HY, Chang YL, Wu CT, Chang GC, Jou YS, Pan SH, Yang PC. *PLoS One*. 2013; 8:e61664. [PubMed: 23626713]
61. Wang Q, Wang L, Li D, Deng J, Zhao Z, He S, Zhang Y, Tu Y. *Cancer Epidemiol*. 2013; 37:79. [PubMed: 22999822]
62. Huang W, Wang J, Zhang D, Chen W, Hou L, Wu X, Lu Y. *Cell Physiol Biochem*. 2015; 37:1659. [PubMed: 26536004]
63. Liang ML, Hsieh TH, Ng KH, Tsai YN, Tsai CF, Chao ME, Liu DJ, Chu SS, Chen W, Liu YR, Liu RS, Lin SC, Ho DM, Wong TT, Yang MH, Wang HW. *Oncotarget*. 2016; 7:19723. [PubMed: 26933822]
64. Li KK, Qi Y, Xia T, Chan AK, Zhang ZY, Aibaidula A, Zhang R, Zhou L, Yao Y, Ng HK. *Lab Invest*. 2017; 97:946. [PubMed: 28504687]
65. Kouprina N, Pavlicek A, Collins NK, Nakano M, Noskov VN, Ohzeki J, Mochida GH, Risinger JJ, Goldsmith P, Gonsior M, Solomon G, Gersch W, Kim JH, Barrett JC, Walsh CA, Jurka J, Masumoto H, Larionov V. *Hum Mol Genet*. 2005; 14:2155. [PubMed: 15972725]
66. Zhong X, Liu L, Zhao A, Pfeifer GP, Xu X. *Cell Cycle*. 2005; 4:1227. [PubMed: 16123590]
67. Higgins J, Midgley C, Bergh AM, Bell SM, Askham JM, Roberts E, Binns RK, Sharif SM, Bennett C, Glover DM, Woods CG, Morrison EE, Bond J. *BMC Cell Biol*. 2010; 11:85. [PubMed: 21044324]
68. do Carmo Avides M, Glover DM. *Science*. 1999; 283:1733. [PubMed: 10073938]
69. Fish JL, Kosodo Y, Enard W, Paabo S, Huttner WB. *Proc Natl Acad Sci U S A*. 2006; 103:10438. [PubMed: 16798874]

70. Pulvers JN, Bryk J, Fish JL, Wilsch-Brauninger M, Arai Y, Schreier D, Naumann R, Helppi J, Habermann B, Vogt J, Nitsch R, Toth A, Enard W, Paabo S, Huttner WB. *Proc Natl Acad Sci U S A*. 2010; 107:16595. [PubMed: 20823249]
71. Williams SE, Garcia I, Crowther AJ, Li S, Stewart A, Liu H, Lough KJ, O'Neill S, Veleta K, Oyarzabal EA, Merrill JR, Shih YY, Gershon TR. *Development*. 2015; 142:3921. [PubMed: 26450969]
72. Bond J, Roberts E, Mochida GH, Hampshire DJ, Scott S, Askham JM, Springell K, Mahadevan M, Crow YJ, Markham AF, Walsh CA, Woods CG. *Nat Genet*. 2002; 32:316. [PubMed: 12355089]
73. Roberts E, Hampshire DJ, Pattison L, Springell K, Jafri H, Corry P, Mannon J, Rashid Y, Crow Y, Bond J, Woods CG. *J Med Genet*. 2002; 39:718. [PubMed: 12362027]
74. Letard P, Drunat S, Vial Y, Duerinckx S, Ernault A, Amram D, Arpin S, Bertoli M, Busa T, Ceulemans B, Desir J, Doco-Fenzy M, Elalaoui SC, Devriendt K, Faivre L, Francannet C, Genevieve D, Gerard M, Gitiaux C, Julia S, Lebon S, Lubala T, Mathieu-Dramard M, Maurey H, Metreau J, Nasserreddine S, Nizon M, Pierquin G, Pouvreau N, Rivier-Ringenbach C, Rossi M, Schaefer E, Sefiani A, Sigaudy S, Sznajer Y, Tunca Y, Guilmin Crepon S, Alberti C, Elmaleh-Berges M, Benzacken B, Wollnick B, Woods CG, Rauch A, Abramowicz M, El Ghouzzi V, Gressens P, Verloes A, Passemard S. *Hum Mutat*. 2017
75. Luers GH, Michels M, Schwaab U, Franz T. *Mech Dev*. 2002; 118:229. [PubMed: 12351193]
76. Fujimori A, Itoh K, Goto S, Hirakawa H, Wang B, Kokubo T, Kito S, Tsukamoto S, Fushiki S. *Brain Dev*. 2014; 36:661. [PubMed: 24220505]
77. Bruning-Richardson A, Bond J, Alsiary R, Richardson J, Cairns DA, McCormack L, Hutson R, Burns P, Wilkinson N, Hall GD, Morrison EE, Bell SM. *Br J Cancer*. 2011; 104:1602. [PubMed: 21505456]
78. Alsiary R, Bruning-Richardson A, Bond J, Morrison EE, Wilkinson N, Bell SM. *PLoS One*. 2014; 9:e97059. [PubMed: 24830737]
79. Horvath S, Zhang B, Carlson M, Lu KV, Zhu S, Felciano RM, Laurance MF, Zhao W, Qi S, Chen Z, Lee Y, Scheck AC, Liao LM, Wu H, Geschwind DH, Febbo PG, Kornblum HI, Cloughesy TF, Nelson SF, Mischel PS. *Proc Natl Acad Sci U S A*. 2006; 103:17402. [PubMed: 17090670]
80. Hagemann C, Anacker J, Gergras S, Kuhnel S, Said HM, Patel R, Kammerer U, Vordermark D, Roosen K, Vince GH. *Oncol Rep*. 2008; 20:301. [PubMed: 18636190]
81. Bikeye SN, Colin C, Marie Y, Vampouille R, Ravassard P, Rousseau A, Boisselier B, Idbaih A, Calvo CF, Leuraud P, Lassalle M, El Hallani S, Delattre JY, Sanson M. *Cancer Cell Int*. 2010; 10:1. [PubMed: 20142996]
82. Kato TA, Okayasu R, Jeggo PA, Fujimori A. *Int J Radiat Biol*. 2011; 87:1189. [PubMed: 21923303]
83. Vulcani-Freitas TM, Saba-Silva N, Cappellano A, Cavalheiro S, Marie SK, Oba-Shinjo SM, Malheiros SM, de Toledo SR. *Childs Nerv Syst*. 2011; 27:71. [PubMed: 20694558]
84. Hussain MS, Baig SM, Neumann S, Peche VS, Szczepanski S, Nurnberg G, Tariq M, Jameel M, Khan TN, Fatima A, Malik NA, Ahmad I, Altmuller J, Frommolt P, Thiele H, Hohne W, Yigit G, Wollnik B, Neubauer BA, Nurnberg P, Noegel AA. *Hum Mol Genet*. 2013; 22:5199. [PubMed: 23918663]
85. Meyerson M, Harlow E. *Mol Cell Biol*. 1994; 14:2077. [PubMed: 8114739]
86. Sherr CJ, Beach D, Shapiro GI. *Cancer Discov*. 2016; 6:353. [PubMed: 26658964]
87. Tigan AS, Bellutti F, Kollmann K, Tebb G, Sexl V. *Oncogene*. 2016; 35:3083. [PubMed: 26500059]
88. Sherr CJ, Roberts JM. *Genes Dev*. 1999; 13:1501. [PubMed: 10385618]
89. Blain SW, Montalvo E, Massague J. *J Biol Chem*. 1997; 272:25863. [PubMed: 9325318]
90. Cheng M, Sexl V, Sherr CJ, Roussel MF. *Proc Natl Acad Sci U S A*. 1998; 95:1091. [PubMed: 9448290]
91. LaBaer J, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, Fattaey A, Harlow E. *Genes Dev*. 1997; 11:847. [PubMed: 9106657]
92. Ruas M, Peters G. *Biochim Biophys Acta*. 1998; 1378:F115. [PubMed: 9823374]

93. Bates S, Bonetta L, MacAllan D, Parry D, Holder A, Dickson C, Peters G. *Oncogene*. 1994; 9:71. [PubMed: 8302605]
94. Lucas JJ, Szepesi A, Modiano JF, Domenico J, Gelfand EW. *J Immunol*. 1995; 154:6275. [PubMed: 7759865]
95. Hu MG, Deshpande A, Enos M, Mao D, Hinds EA, Hu GF, Chang R, Guo Z, Dose M, Mao C, Tsiachlis PN, Gounari F, Hinds PW. *Cancer Res*. 2009; 69:810. [PubMed: 19155308]
96. Malumbres M, Sotillo R, Santamaria D, Galan J, Cerezo A, Ortega S, Dubus P, Barbacid M. *Cell*. 2004; 118:493. [PubMed: 15315761]
97. Rane SG, Dubus P, Mettus RV, Galbreath EJ, Boden G, Reddy EP, Barbacid M. *Nat Genet*. 1999; 22:44. [PubMed: 10319860]
98. Tsutsui T, Hesabi B, Moons DS, Pandolfi PP, Hansel KS, Koff A, Kiyokawa H. *Mol Cell Biol*. 1999; 19:7011. [PubMed: 10490638]
99. Nagasawa M, Gelfand EW, Lucas JJ. *Oncogene*. 2001; 20:2889. [PubMed: 11420701]
100. Ferguson KL, Callaghan SM, O'Hare MJ, Park DS, Slack RS. *J Biol Chem*. 2000; 275:33593. [PubMed: 10915795]
101. Calegari F, Huttner WB. *J Cell Sci*. 2003; 116:4947. [PubMed: 14625388]
102. Beukelaers P, Vandenbosch R, Caron N, Nguyen L, Belachew S, Moonen G, Kiyokawa H, Barbacid M, Santamaria D, Malgrange B. *Stem Cells*. 2011; 29:713. [PubMed: 21319271]
103. Ericson KK, Krull D, Slomiany P, Grossel MJ. *Mol Cancer Res*. 2003; 1:654. [PubMed: 12861051]
104. Tadesse S, Yu M, Kumarasiri M, Le BT, Wang S. *Cell Cycle*. 2015; 14:3220. [PubMed: 26315616]
105. Costello JF, Plass C, Arap W, Chapman VM, Held WA, Berger MS, Su Huang HJ, Cavenee WK. *Cancer Res*. 1997; 57:1250. [PubMed: 9102208]
106. Lam PY, Di Tomaso E, Ng HK, Pang JC, Roussel MF, Hjelm NM. *Br J Neurosurg*. 2000; 14:28. [PubMed: 10884881]
107. Mendrzyk F, Radlwimmer B, Joos S, Kokocinski F, Benner A, Stange DE, Neben K, Fiegler H, Carter NP, Reifemberger G, Korshunov A, Lichter P. *J Clin Oncol*. 2005; 23:8853. [PubMed: 16314645]
108. Chen SM, Chen HC, Chen SJ, Huang CY, Chen PY, Wu TW, Feng LY, Tsai HC, Lui TN, Hsueh C, Wei KC. *World J Surg Oncol*. 2013; 11:87. [PubMed: 23594394]
109. Li B, He H, Tao BB, Zhao ZY, Hu GH, Luo C, Chen JX, Ding XH, Sheng P, Dong Y, Zhang L, Lu YC. *Oncol Rep*. 2012; 28:909. [PubMed: 22736304]
110. Li X, Gong X, Chen J, Zhang J, Sun J, Guo M. *Biochem Biophys Res Commun*. 2015; 460:670. [PubMed: 25817794]
111. Bellail AC, Olson JJ, Hao C. *Nat Commun*. 2014; 5:4234. [PubMed: 24953629]
112. Whiteway SL, Harris PS, Venkataraman S, Alimova I, Birks DK, Donson AM, Foreman NK, Vibhakkar R. *J Neurooncol*. 2013; 111:113. [PubMed: 23138228]
113. Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. *Nat Rev Drug Discov*. 2015; 14:130. [PubMed: 25633797]
114. Vaughn DJ, Hwang WT, Lal P, Rosen MA, Gallagher M, O'Dwyer PJ. *Cancer*. 2015; 121:1463. [PubMed: 25522918]
115. Taylor JW, Molinaro AM, Phillips JJ, James CD, Butowski NA, Clarke JL, Oberheim Bush N, Chang SM, Berger MS, Prados M. *Neuro Oncol*. 2017; 19:iii83.
116. Gururangan S, Becher O, Leary S, Onar-Thomas A, Huang J, Phillips J, Stewart CF, Fangusaro J, Lulla R, Baxter P, Pollack I, Boyett J, Fouladi M. *Neuro Oncol*. 2016; 18:iii28.
117. Patnaik A, Rosen LS, Tolaney SM, Tolcher AW, Goldman JW, Gandhi L, Papadopoulos KP, Beeram M, Rasco DW, Hilton JF, Nasir A, Beckmann RP, Schade AE, Fulford AD, Nguyen TS, Martinez R, Kulanthaivel P, Li LQ, Frenzel M, Cronier DM, Chan EM, Flaherty KT, Wen PY, Shapiro GI. *Cancer Discov*. 2016; 6:740. [PubMed: 27217383]
118. Cook Sangar ML, Genovesi LA, Nakamoto MW, Davis MJ, Knobluagh SE, Ji P, Millar A, Wainwright BJ, Olson JM. *Clin Cancer Res*. 2017; 23:5802. [PubMed: 28637687]
119. Abraham RT. *Genes Dev*. 2001; 15:2177. [PubMed: 11544175]

120. Cimprich KA, Cortez D. Nature reviews. Molecular cell biology. 2008; 9:616. [PubMed: 18594563]
121. Shiloh Y. Current opinion in genetics & development. 2001; 11:71. [PubMed: 11163154]
122. Shiloh Y. Nat Rev Cancer. 2003; 3:155. [PubMed: 12612651]
123. Germann SM, Schramke V, Pedersen RT, Gallina I, Eckert-Boulet N, Oestergaard VH, Lisby M. J Cell Biol. 2014; 204:45. [PubMed: 24379413]
124. Gray S, Allison RM, Garcia V, Goldman AS, Neale MJ. Open biology. 2013; 3:130019. [PubMed: 23902647]
125. Smith J, Tho LM, Xu N, Gillespie DA. Advances in cancer research. 2010; 108:73. [PubMed: 21034966]
126. Yang J, Yu Y, Hamrick HE, Duerksen-Hughes PJ. Carcinogenesis. 2003; 24:1571. [PubMed: 12919958]
127. Heffernan TP, Kawasumi M, Blasina A, Anderes K, Conney AH, Nghiem P. The Journal of investigative dermatology. 2009; 129:1805. [PubMed: 19242509]
128. Myers K, Gagou ME, Zuazua-Villar P, Rodriguez R, Meuth M. PLoS genetics. 2009; 5:e1000324. [PubMed: 19119425]
129. Lee Y, Shull ER, Frappart PO, Katyal S, Enriquez-Rios V, Zhao J, Russell HR, Brown EJ, McKinnon PJ. The EMBO journal. 2012; 31:1177. [PubMed: 22266795]
130. Offer H, Erez N, Zurer I, Tang X, Milyavsky M, Goldfinger N, Rotter V. Carcinogenesis. 2002; 23:1025. [PubMed: 12082025]
131. Paulsen RD, Cimprich KA. DNA repair. 2007; 6:953. [PubMed: 17531546]
132. Brown EJ, Baltimore D. Genes Dev. 2000; 14:397. [PubMed: 10691732]
133. Carletti B, Rossi F. The Neuroscientist: a review journal bringing neurobiology, neurology and psychiatry. 2008; 14:91.
134. Lang PY, Nanjangud GJ, Sokolsky-Papkov M, Shaw C, Hwang D, Parker JS, Kabanov AV, Gershon TR. Development. 2016; 143:4038. [PubMed: 27803059]
135. Goodship J, Gill H, Carter J, Jackson A, Splitt M, Wright M. Am J Hum Genet. 2000; 67:498. [PubMed: 10889046]
136. O'Driscoll M, Ruiz-Perez VL, Woods CG, Jeggo PA, Goodship JA. Nat Genet. 2003; 33:497. [PubMed: 12640452]
137. Tanaka A, Weinl S, Nagy N, O'Driscoll M, Lai-Cheong JE, Kulp-Shorten CL, Knable A, Carpenter G, Fisher SA, Hiragun M, Yanase Y, Hide M, Callen J, McGrath JA. Am J Hum Genet. 2012; 90:511. [PubMed: 22341969]
138. Amakye D, Jagani Z, Dorsch M. Nature medicine. 2013; 19:1410.
139. Wechsler-Reya R, Scott MP. Annual review of neuroscience. 2001; 24:385.
140. Mao J, Ligon KL, Rakhlin EY, Thayer SP, Bronson RT, Rowitch D, McMahon AP. Cancer Res. 2006; 66:10171. [PubMed: 17047082]
141. Kool M, Jones DT, Jager N, Northcott PA, Pugh TJ, Hovestadt V, Piro RM, Esparza LA, Markant SL, Remke M, Milde T, Bourdeaut F, Ryzhova M, Sturm D, Pfaff E, Stark S, Hutter S, Seker-Cin H, Johann P, Bender S, Schmidt C, Rausch T, Shih D, Reimand J, Sieber L, Wittmann A, Linke L, Witt H, Weber UD, Zapatka M, Konig R, Beroukheim R, Bergthold G, van Sluis P, Volckmann R, Koster J, Versteeg R, Schmidt S, Wolf S, Lawerenz C, Bartholomae CC, von Kalle C, Unterberg A, Herold-Mende C, Hofer S, Kulozik AE, von Deimling A, Scheurlen W, Felsberg J, Reifenberger G, Hasselblatt M, Crawford JR, Grant GA, Jabado N, Perry A, Cowdrey C, Croul S, Zadeh G, Korbel JO, Doz F, Delattre O, Bader GD, McCabe MG, Collins VP, Kieran MW, Cho YJ, Pomeroy SL, Witt O, Brors B, Taylor MD, Schuller U, Korshunov A, Eils R, Wechsler-Reya RJ, Lichter P, Pfister SM. IPT Project. Cancer Cell. 2014; 25:393. [PubMed: 24651015]
142. Herr I, Debatin KM. Blood. 2001; 98:2603. [PubMed: 11675328]
143. Roos WP, Kaina B. Trends in molecular medicine. 2006; 12:440. [PubMed: 16899408]
144. Barnett GC, West CM, Dunning AM, Elliott RM, Coles CE, Pharoah PD, Burnet NG. Nat Rev Cancer. 2009; 9:134. [PubMed: 19148183]
145. Lang PY, Nanjangud GJ, Sokolsky-Papkov M, Shaw C, Hwang D, Parker JS, Kabanov AV, Gershon TR. Development. 2016 In Press.

146. Thomas A, Redon CE, Sciuto L, Padiernos E, Ji J, Lee MJ, Yuno A, Lee S, Zhang Y, Tran L, Yutzy W, Rajan A, Guha U, Chen H, Hassan R, Alewine CC, Szabo E, Bates SE, Kinders RJ, Steinberg SM, Doroshow JH, Aladjem MI, Trepel JB, Pommier Y. *J Clin Oncol*. 2017 JCO2017766915.
147. Di Benedetto A, Ercolani C, Mottolese M, Sperati F, Pizzuti L, Vici P, Terrenato I, Shaaban AM, Humphries MP, Di Lauro L, Barba M, Vitale I, Ciliberto G, Speirs V, De Maria R, Maugeri-Sacca M. *Sci Rep*. 2017; 7:8078. [PubMed: 28808232]
148. Kemp MG, Sancar A. *J Biol Chem*. 2016; 291:9330. [PubMed: 26940878]
149. Ruzankina Y, Pinzon-Guzman C, Asare A, Ong T, Pontano L, Cotsarelis G, Zediak VP, Velez M, Bhandoola A, Brown EJ. *Cell Stem Cell*. 2007; 1:113. [PubMed: 18371340]
150. Onksen JL, Brown EJ, Blendy JA. *Neuropsychopharmacology*. 2011; 36:960. [PubMed: 21248719]
151. Dean JL, McClendon AK, Knudsen ES. *J Biol Chem*. 2012; 287:29075. [PubMed: 22733811]
152. Roberts PJ, Bisi JE, Strum JC, Combest AJ, Darr DB, Usary JE, Zamboni WC, Wong KK, Perou CM, Sharpless NE. *J Natl Cancer Inst*. 2012; 104:476. [PubMed: 22302033]
153. Packer RJ, Gajjar A, Vezina G, Rorke-Adams L, Burger PC, Robertson PL, Bayer L, LaFond D, Donahue BR, Marymont MH, Muraszko K, Langston J, Spoto R. *J Clin Oncol*. 2006; 24:4202. [PubMed: 16943538]
154. Sawin KE, Mitchison TJ. *Proc Natl Acad Sci U S A*. 1995; 92:4289. [PubMed: 7753799]
155. Faheem M, Naseer MI, Rasool M, Chaudhary AG, Kumosani TA, Ilyas AM, Pushparaj P, Ahmed F, Algahtani HA, Al-Qahtani MH, Saleh Jamal H. *BMC Med Genomics*. 2015; 8(Suppl 1):S4.
156. Komlodi-Pasztor E, Sackett DL, Fojo AT. *Clin Cancer Res*. 2012; 18:51. [PubMed: 22215906]
157. Ito S, Hoshino T, Prados MD, Edwards MS. *Cancer*. 1992; 70:671. [PubMed: 1623482]
158. Stensjoen AL, Solheim O, Kvistad KA, Haberg AK, Salvesen O, Berntsen EM. *Neuro Oncol*. 2015; 17:1402. [PubMed: 25758748]

Figure 1.

The roles of specific microcephaly proteins in normal proliferation and the effects of their disruption. (A) EG5 (blue and tan coils) is a plus-end-directed microtubule motor protein that pushes apart the duplicated centrosomes to create a bipolar mitotic spindle in prophase and prometaphase, allowing for separation of sister chromatids. (A') In the absence of EG5 activity, the centrosomes fail to separate, resulting in the formation of a monoaster, which triggers mitotic arrest that can lead to apoptosis. (B) ASPM (green octagon) is a microtubule minus-end-associated protein found at mitotic spindle poles that has been implicated in both mediating symmetric cell divisions (indicated by same colored cells) and orienting the cell division plane (dotted line) in neural stem and progenitor cells. (B') The absence of ASPM creates more asymmetric divisions (indicated by different colored cells) and altered division planes (top vs. bottom), both of which can promote differentiation over continued proliferation. (C) CDK6 (red octagon) associates with mitotic centrosomes in the cell's (purple) nucleus (blue), where it may mediate centrosome duplication, spindle assembly, or cellular motility (indicated by dotted lines and arrows). (C') The *CDK6* mutation associated with microcephalic patients did not reduce CDK6 (grey polygon) protein levels but rather altered its function and behavior such that it no longer associated with centrosomes, which

led to supernumerary centrosomes, inappropriate mitotic spindle alignment, abnormal cellular polarity, and reduced cell motility. (D) Endogenous, replication-associated DNA damage (lightning bolt) activates the DNA damage response protein ATR, which recognizes single-stranded DNA coated by RPA (orange circles) at stalled replication forks. Activated ATR can promote G₂/M arrest by barring mitotic entry through preventing CDK1/CDC2 activation and by targeting CEP63 to block assembly of the mitotic spindle. (D') Absence of ATR function limits DNA repair while also permitting CGNPs with DNA damage to enter mitosis. Progression through mitosis exacerbates DNA damage leading to severe chromosomal aberrations. Overwhelming DNA damage causes ATM to activate p53, resulting in widespread apoptosis of CGNPs.

Table 1

Several KIF11/EG5 inhibitors were tested in clinical trials for a diverse range of non-primary brain cancers, with only one currently active trial for multiple myeloma.

Compound	Cancer	Combination	Phase*	Study start	Outcomes; Dose-limiting toxicities
Ispinesib (SB-715992)	Recurrent head & neck cancer	None	II (C)	2004	No benefit; neutropenia and infection toxicities
	AML, ALL, CML, or MDS	None	I (C)	2004	Unavailable
	Metastatic prostate cancer	None	II (C)	2004	Drug failure [29]
	Metastatic malignant melanoma	None	II (C)	2004	35% subjects with stable disease; well-tolerated [30]
	Metastatic liver cancer	None	II (C)	2004	46% subjects with stable disease; granulocytopenia toxicity [31]
	Breast cancer	None	II (C)	2004	Unavailable
	Relapsed ovarian cancer	None	II (C)	2004	45% subjects with stable disease; neutropenia toxicity [32]
	Metastatic NSCLC	None	II (C)	2004	Unavailable
	Solid tumors	Capecitabine, Carboplatin, or Docetaxel	I (C)	2005	29% subjects with stable disease; neutropenia toxicity [33]
	Metastatic colorectal cancer	None	II (C)	2005	Neutropenia toxicity
SB-743921	Metastatic kidney cancer	None	II (C)	2006	30% subjects with stable disease; neutropenia, hyperuricemia toxicities [34]
	Refractory solid tumors or lymphomas	None	I (C)	2006	Unavailable
	Advanced/metastatic breast cancer	None	I/II (C)	2008	7% subjects with partial response; neutropenia toxicity [35]
	Recurrent solid tumors or lymphomas	None	I (C)	2005	Neutropenia toxicity [36]
Litronesib (LY-2523355)	NHL	None	I/II (C)	2006	Hematological toxicities [37]
	SCLC	None	II (C)	2009	5% subjects with progression-free survival; neutropenia toxicity
	All advanced/metastatic solid cancers	Pegfilgrastim	I (C)	2010	2% subjects with partial response; neutropenia toxicity [38]
	Ovarian, NSCLC, prostate, colorectal, gastroesophageal, or head & neck cancer	Pegfilgrastim	II (C)	2010	1% subjects with progression-free survival; neutropenia toxicity
	Acute leukemia	None	I (T)	2010	Unavailable
	Advanced/metastatic solid cancer	None	I (C)	2011	No benefit; neutropenia toxicity [39]
	Metastatic/recurrent breast cancer	(Peg)filgrastim	II (C)	2011	2% subjects with progression-free survival; neutropenia toxicity

Compound	Cancer	Combination	Phase*	Study start	Outcomes; Dose-limiting toxicities
Filanesib (ARRY-520)	Advanced solid tumors	Filgrastim	I (C)	2007	18% subjects with stable disease; neutropenia toxicity ^[40]
	AML or MDS	None	I/II (C)	2008	Unavailable
	MM or PCL	Dexamethasone & Filgrastim	I/II (C)	2009	Unavailable
	MM or PCL	Bortezomib, Dexamethasone, & Filgrastim	I (C)	2010	Unavailable
	MM or PCL	Carfilzomib, Dexamethasone, & Filgrastim	I (C)	2011	Unavailable
	Advanced MM	Carfilzomib, Dexamethasone, & Filgrastim	II (C)	2013	Unavailable
	Advanced MM	Filgrastim	II (C)	2014	Unavailable
	Relapsed/refractory MM	Pomalidomide & Dexamethasone	I/II	2015	Neutropenia toxicity ^[41]
	Advanced solid tumors or lymphomas	None	I (C)	2010	28% subjects with 6-week stable disease; neutropenia toxicity ^[42]
	Advanced solid tumors or lymphomas	None	I (T)	2007	Well-tolerated ^[43]
AZD-4877	AML	None	I (T)	2007	No benefit; stomatitis and hyperbilirubinemia toxicities ^[44]
	Recurrent AML, ALL, NHL, or MM	None	I (T)	2007	Unavailable
	Advanced bladder cancer	None	II (C)	2008	3% subjects with partial response, 18% with stable disease; neutropenia toxicity ^[45]
MK-0731	Advanced solid tumors	None	I (C)	2005	Extended stable disease; neutropenia toxicity ^[46]
ARQ-621	Advanced solid or hematologic cancers	None	I (C)	2009	Anemia, sepsis, pneumonia, pleural effusion, thrombosis toxicities ^[47]

Information from clinicaltrials.gov: accessed 12/17/17.

* Indicates current active phase or most recently reported completed phase. C = completed, T = terminated.

Table 2

Three CDK6 inhibitors are in active clinical trials for CNS cancers.

Compound	Cancer	Combination	Phase*	Study start	Outcomes; Dose-limiting toxicities
Palbociclib (PD-0332991)	RB ⁺ CNS germ cell tumor	None	II	2009	28% with 24-week progression-free survival; hematological toxicity [114]
	Recurrent RB ⁺ glioblastoma	None	II (T)	2010	No benefit [115]
	Recurrent, refractory, or progressive RB ⁺ CNS tumors in young patients	None	I	2014	Neutropenia/lymphopenia toxicities [116]
	Recurrent oligodendroglioma or oligoastrocytoma	None	II	2015	Unavailable
	HER2 ⁺ breast cancer w/brain metastasis	Trastuzumab	II	2016	Unavailable
	Metastatic malignant neoplasm to brain	None	II	2016	Unavailable
Ribociclib (LEE-011)	High grade gliomas after radiation	XRT	I/II	2016	Unavailable
	Recurrent glioblastoma or anaplastic glioma	None	I	2016	Unavailable
	Preoperative glioma and meningioma	None	I	2016	Unavailable
Abemaciclib (LY-2835219)	Solid tumors, including glioblastoma	None	I	2009	18% glioblastoma subjects achieved stable disease; fatigue toxicity [117]
	Diffuse intrinsic pontine glioma	None	I	2015	Unavailable
	Recurrent brain tumors	None	II	2017	Unavailable
	Recurrent glioblastoma	None	II	2017	Unavailable

Information from clinicaltrials.gov: accessed 12/17/17.

* Indicates current active phase or most recently reported completed phase. S: suspended, T: terminated.

Table 3

Three ATR inhibitors are in active clinical trials, but not for primary brain malignancies.

Compound	Cancer	Combination	Phase*	Study start	Outcomes; Dose-limiting toxicities
VX-970 (VE-822, M6620)	Advanced solid tumors	Gemcitabine	I	2012	Unavailable
	Advanced solid tumors	None	I	2014	Unavailable
	Uterine/cervical cancer, neuroendocrine tumor, NSCLC, SCLC, ovarian cancer	Topotecan	I/II	2015	Unavailable
	Head & neck squamous cell carcinoma	Radiation & Cisplatin	I	2016	Unavailable
	Metastatic urothelial cancer	Gemcitabine & Cisplatin	II	2016	Unavailable
	Advanced/metastatic solid tumors	Topotecan	I	2016	10% subjects with partial response, 33% with stable disease; hematological toxicities [146]
	Advanced/metastatic solid tumors	Irinotecan	I	2016	Unavailable
	Recurrent ovarian, primary peritoneal, or fallopian tube cancer	Gemcitabine &/or Carboplatin	I/II	2016	Unavailable
	Brain metastases from NSCLC, SCLC, or neuroendocrine tumors	XRT	I	2016	Unavailable
	Refractory solid tumors	Veliparib & Cisplatin	I	2016	Unavailable
AZD-6738	Relapsed/refractory CLL, PLL, or B-cell lymphoma	None	I	2013	Unavailable
	Advanced solid tumors	Carboplatin, Olaparib, or MEDI-4736	I/II	2014	Unavailable
	Metastatic NSCLC	Durvalumab	II	2017	Unavailable
BAY1895344	Advanced solid tumors/lymphomas	None	I	2017	Unavailable

Information from clinicaltrials.gov: accessed 12/17/17.

* Indicates current active phase or most recently reported completed phase.