Radiotherapy Followed by Aurora Kinase Inhibition Targets Tumor-Propagating Cells in Human Glioblastoma

Nan Li1,2,3, Dustin J. Maly4, Yvan H. Chanterey1,2,5, Daniel W. Sirkis1,2,5, Jean L. Nakamura5,6, Mitchel S. Berger2,5, C. David James2,5, Kevan M. Shokat7, William A. Weiss1,2,5, and Anders I. Persson1,2,3

Abstract

Glioblastoma (GBM) is the most common malignant primary brain tumor. Radiotherapy fails to eliminate subpopulations of stem-like tumor-propagating cells (TPC), resulting in tumor regrowth. To identify kinases that promote TPC self-renewal rather than increasing proliferation in human GBM cultures, we screened a library of 54 nonselective tool compounds and determined their kinase inhibitor profiles in vitro. Most compounds inhibited aurora kinase (AURK) activity and blocked TPC self-renewal, while inducing GBM cell polynucleation and apoptosis. To prevent regrowth by TPCs, we used a priming dose of radiation followed by incubation with the pan-AURK inhibitor VX680 to block self-renewal and induce apoptosis in GBM cultures. In mice xenografted with human GBM cells, radiotherapy followed by VX680 treatment resulted in reduced tumor growth and increased survival relative to either monotherapy alone or VX680 treatment before radiation. Our results indicate that AURK inhibition, subsequent to radiation, may enhance the efficacy of radiotherapy by targeting radioresistant TPCs in human GBMs.

Introduction

Conventional therapy of patients with glioblastoma (GBM) includes surgical removal of the tumor followed by radiotherapy and treatment with alkylating agents. Despite this multimodal treatment approach, tumor regrowth is observed in nearly all cases, with patients usually succumbing to the disease within 6 to 12 months (1). Failure to improve upon this outcome is primarily a consequence of inherent intratumoral cellular and molecular heterogeneity, which confers upon GBM the ability to evolve during treatment (2–4). Among the subpopulation of cells in GBM are highly tumorigenic and treatment-resistant tumor-propagating cells (TPC) that are thought to be especially important in repopulating tumors following treatment (5–8). One such subpopulation, expressing the cell-surface antigen prominin 1 (PROM1, CD133), has been shown to be highly tumorigenic following intracranial transplantation into immunocompromised NOD-SCID mice (9, 10), and CD133+ cells have additionally demonstrated radioresistance as well as reduced sensitivity to the DNA alkylator temozolomide (9, 11). In murine GBMs originating from subventricular zone (SVZ) neural stem cells (NSC), a subpopulation of slow-dividing nestin-expressing tumor cells show temozolomide resistance and underlie regrowth (12). Label-retention studies in vitro show that CD133+ TPCs from human primary GBMs are slow dividing (13), which, in turn, is thought to be a key factor that contributes to CD133+ cell therapy resistance. In patients with GBM, a high proliferative index for CD133+ tumor cells is associated with a very poor survival (14). In addition, cell cycling of CD133+ GBM cells is influenced by the tumor microenvironment (15), which also evolves in association with treatment. Recent studies have identified many alternative TPC markers in human GBM (16). The cell surfaceialoglycoprotein podoplanin (PDPN) shows partial coexpression with CD133 in GBM cultures and tissues (15, 17). A better understanding of the intrinsic and extrinsic factors that regulate TPC cell cycling in GBM is important for achieving improved outcomes for patients with GBM.

Sequencing of the human genome has revealed more than 500 protein kinases in mammalian cells, that activity of which influence a variety of cellular processes, including metabolism, transcription, cell-cycle progression, apoptosis, motility, and differentiation (18). Refinement of kinase small-molecule inhibitors has led to the development of compounds with highly selective activities for specific kinase, and such compounds have been used in numerous clinical trials for treating patients with GBM. Other inhibitors, such as imatinib mesylate, sunitinib, and sorafenib, display multikinase profiles, and have shown efficacy in treating chronic myelogenous leukemia, gastrointestinal stromal tumor, and hepatocellular carcinoma, respectively (19–21). Several protein kinase inhibitors have been identified that reduce GBM TPC
self-renewal and inhibit TPC tumorigenicity (22–24), though the use of these inhibitors, as monotherapies, ultimately fails due to therapy-resistant subpopulation expansion.

To identify kinases that govern self-renewal capacity in GBM tumorsphere cultures, we synthesized a library of 54 structurally related tool compounds and determined their multitargeted kinase inhibition profiles by screening against 40 kinases, using the Ambit kinase platform (25). In testing these compounds against three human GBM cultures, and determined that aurora kinase (AURK) is especially important for TPC self-renewal. This observation prompted our use of the pan-AURK inhibitor VX680, in reducing TPC self-renewal after radiation treatment. In cell culture, as well as in xenograft models, we found that radiation followed by VX680 more effectively induced apoptosis and reduced tumor growth, as compared with either monotherapy. Our results support small-molecule inhibitor targeting of GBM TPCs, subsequent to radiotherapy, for improving radiation antitumor effects, and for improving GBM patient outcomes.

Materials and Methods

Cell culture

Tumor tissue was provided by UCSF’s Brain Tumor Research Center Tissue Bank and acquired from biopsies of human patients with GBM. The samples were collected during surgery from patients given consent and deidentified according to the protocol approved by the UCSF Committee on Human Research. Two patient–derived GBMs (SF6969 and SF7192) acquired at UCSF (in 2008, DNA fingerprinted 2009) were dissociated using papain, and cell cultures were established using neurobasal (NBE) media (–A; Invitrogen) supplemented with 1% v/v B27 supplement, 0.5% v/v N2 supplement, 20 ng/mL FGF-2 (Peprotech), 20 ng/mL EGF (Sigma-Aldrich), 2 mmol/L L-glutamine, penicillin/streptomycin, and incubated at 37°C in 5% CO₂. From UCSF investigator David James, we received (in 2010) the well-characterized GS2 GBM cells (26) and primary GBM43 cells (27). Tumorspheres were passaged using enzymatic dissociation with Accutase (Innovative Cell Technologies).

Chemical synthesis and validation of kinase inhibition profiles

A library of 54 multitargeted small-molecule kinase tool compounds was synthesized to generate compounds with diverse serine/threonine and tyrosine kinase selectivity profiles. To identify the kinase selectivity profiles of the generated tool compounds, the KINOMEScan binding assay (consisting of 40 kinases) was used (DiscoverRX). All compounds were dissolved in DMSO at an initial concentration of 10 mmol/L.

Proliferation assay

To assess the antiproliferative effects of tool compounds or combinations of VX680 treatment and γ-irradiation, we used 96-well polyornithine/laminin–coated plates, plated 0.3 × 10⁵ GBM cells (6969, GS2, 7192, and GBM43) per well in NBE media, and added 0.1, 1, or 10 μmol/L concentration of each synthesized tool compound, VX680, or DMSO (control). The DNA content was analyzed after 72 hours using the Cyquant NF proliferation assay (Invitrogen). The assay was performed as previously described (28). In brief, cells were lysed and simultaneously incubated at 37°C with a fluorescent probe to label nonfragmented DNA as an indirect measure of the number of cells. The fluorescence intensity was measured at Ex/Em + 485/530 nm using a Tecan microplate reader. A standard curve was generated by plotting the number of plated cells (1,000–40,000) against corresponding fluorescent values, resulting in the equation  

\[ y = x + 150, R^2 = 1. \]

The number of cells in each sample were calculated using this equation. Experiments were done in triplicates.

Self-renewal assay

To assess sphere-forming ability, tumor cells were plated at 1 × 10⁴ cells/mL in ultralow- adherent 24-well plates. The following day, GBM cells (6969, GS2, 7192, and GBM43) were incubated with 10 μmol/L of each tool compound or DMSO (control) for 72 hours in NBE media, dissociated with Accutase, and then 200 cells were plated as single cells per well in ultralow-adherent 96-well plates. Ten days later, we quantified the number of secondary spheres. Experiments were performed in triplicates from three separate passages.

Aptoptosis assay

Using 96-well polyornithine/laminin–coated plates, we plated 0.3 × 10⁵ 6969 GBM cells (or GBM43 cells) per well in NBE media and added 0.1, 1, or 10 μmol/L concentration of each synthesized tool compound or DMSO (control). As a positive control, we used camptothecin. As a negative control, we used the caspase-3/7 inhibitor Ac-DEVD-CHO. The assay was performed according to instructions from the manufacturer (Senzolyte Homogenous AFC Caspase-3/7 assay kit; Anaspec). In brief, the GBM cells were cultured for 48 hours and 50 mL/well of caspase-3/7 substrate solution was added to each well. The plates were placed on a shaker at room temperature and the fluorescence intensity was measured 30 minutes later at Ex/Eth + 380/500 nm using a Tecan microplate reader. The relative intensities were calculated versus DMSO control. Experiments were done in triplicates.

Immunocytochemistry

Cells were cultured on polyornithine/laminin–coated plates at a density of 1 × 10⁴ cells/cm². Cultured cells were fixed in 4% paraformaldehyde for 10 minutes at 4°C, washed in PBS, and incubated in PBS*(PBS containing 5% donkey serum, 3% BSA, 0.3% Triton-X100) for 30 minutes. Primary antibodies against Ser-10 phosphorylated histone H3 (1:1,000; Millipore) and nestin (1:200; Millipore) were incubated in PBS* overnight at 4°C. Cells were washed and incubated with anti-mouse Alexa 488 and anti-rabbit Alexa 594 antibodies (1:1,000; both from Invitrogen) for 2 hours at room temperature. After 3 × PBS (DAPI was included in the second wash), coverslips were mounted using Aquamount (Polysciences). Cells were counted using a Nikon Eclipse E800 fluorescence microscope. The percentage of immunoreactive cells was scored by counting the number of immunoreactive cells in 10 unbiased areas (based on DAPI) in triplicates. Images were taken using a Nikon Eclipse E800 fluorescence microscope.

Immunoblotting

Membranes were blocked with antibodies directed against Ser-10 phosphorylated histone H3 (1:500) and GAPDH (1:5000) from Millipore, histone H3 (1:1000), c-ABL (1:1000), FLT3 (1:1000), AURKA (1:1000), AURKB (1:1000) from Cell Signaling Technology, AURKC (1:200; Zymed), and β-tubulin (1:3000; Calbiochem). Bound antibodies were detected with horseradish peroxidase–linked antibodies against mouse or rabbit IgG (Amer- sham), followed by ECL (Amer- sham).
Fluorescence-activated cell sorting

For all three human GBMs (6969, GS2, and 7192), we dissociated tumorspheres using Accutase, washed cells twice in AutoMACS rinsing buffer (Miltenyi Biotech.), and then incubated human GBM cells with rat PDPN-PE (phycoerythrin; 1:50; AngioBio) and CD133-APC (1:20, clone 293C; Miltenyi Biotech.) antibodies for 30 minutes at 4°C in rinsing buffer and FCR human blocking reagent (1:10; Miltenyi Biotech.). The cells were washed twice in rinsing buffer and the cells were analyzed with the flow cytometer. For in vivo experiments, we gave an i.p. injection of bromodeoxyuridine (BrdUrd; 50 mg/kg; Sigma-Aldrich) 2 hours before isolation and dissociation of enhanced GFP (EGFP)-labeled GBM43 tumors. Cells were rinsed in buffer and incubated with PDPN-PE antibody for 30 minutes at 4°C before cells were fixed and processed for BrdUrd–APC and 7-aminoactinomycin D according to the manufacturer’s protocol (APC-BrdU Flow Kit; BD Biosciences) before analysis using a FACS Calibur flow cytometer (BD Biosciences). As controls, primary antibodies were replaced by isotype-matched antibodies from the same manufacturers. Calculations and preparation of images were performed using FlowJo version 7.6.5 software (Treestar Inc.).

For analysis of cell-cycle phases and apoptosis, cells were dissociated with Accutase, fixed in 70% ethanol at −4°C overnight, nuclei were stained with 5 μg/mL propidium iodide containing 125 U/mL RNase for 30 minutes and analyzed with a FACS Calibur flow cytometer (Becton Dickinson). DNA histograms were modeled of fluorescence and overall survival, OS), we used one-way ANOVA followed by the Newman-Keul post hoc test. Sphere-formation assay and proliferation experiments in vitro, we used one-way ANOVA followed by the Tukey post hoc test.

Results

Identifying self-renewing and radioresistant TPCs in human GBM

Previously described human primary GBMs (SF6969 and SF7192) from UCSF, and a secondary GBM GS2 cell line were cultured as tumorspheres in defined media supplemented with growth factors (Fig. 1A; refs. 29, 30). CD133 has previously been suggested as a marker of TPCs in GBM (10). Similar to CD133, previous studies show that hypoxia induces expression of the stem cell marker PDPN, a cell-surface protein associated with increasing grade and worse prognosis in glioma (17, 31). Flow-cytometry experiments demonstrated that subpopulations in all three GBM cultures expressed PDPN and CD133 (Fig. 1B). To test whether PDPN, similar to CD133 (9), labels radioresistant cells, we exposed cells to a single dose of γ-irradiation (Fig. 2A), sorted on the basis of both kinase targets and compounds. The inhibition scale ranges from 0 to 100 (percentage of, as opposed to the control), and the corresponding color scale is white to blue. For in vivo studies (bioluminescence and overall survival, OS), we used one-way ANOVA followed by the Newman-Keul post hoc test. Sphere-formation assays and proliferation experiments in vitro, we used one-way ANOVA followed by the Tukey post hoc test.

Aurora kinases as therapeutic targets on TPCs in human GBM

The kinase target selectivity profiles of 54 synthesized and structurally related tool compounds were determined using the KINOMEscan screening platform (Fig. 2A). Among other
targets, most of the structurally related compounds effectively inhibited AURK activity. To measure activity against bulk tumor cells, we incubated adherent primary human GBM cells (SF6969, SF7192, and GS2) with each kinase inhibitor (0.1–10 μmol/L, 3d), and quantified proliferation compared with DMSO treated cultures (Fig. 2B). Many compounds inhibited proliferation in a concentration-dependent manner with DMSO treated cultures (Fig. 2B). Many compounds enhanced (P < 0.05 at 1–10 μmol/L cleaved caspase-3/7 activity in 6969 GBM cells compared with DMSO controls (Supplementary Fig. S1A). Although the majority of tumor bulk GBM cells undergo rapid proliferation, an indirect measure of TPCs can be quantified using the secondary tumorsphere formation assay. As a measure of self-renewing TPCs, we incubated tumorspheres with (10 μmol/L 3 days), replated dissociated single cells, and scored secondary tumorspheres 10 days later (Fig. 2B). Surprisingly, we found that most compounds blocked self-renewal of TPCs (Fig. 2B). Intriguingly, the structural features of the tool compounds and the inhibitory profiles of kinase activity (Fig. 2A) were reminiscent of the pan-AURK inhibitor VX680 (32). We therefore compared the effects of VX680 and nine structurally related tool compounds (ASC66-73) on proliferation, self-renewal, and apoptosis in human GBM cultures (data not shown). Using well-characterized human GBM43 cells, we confirmed that two tool compounds and VX680 increased (P < 0.01) apoptosis compared with DMSO treated cultures (Supplementary Fig. S1B). Quantification showed a concentration-dependent inhibitory effect on VX680 that was less stringent for ASC66 (Fig. 2C and D). Albeit both ASC66 and VX680 reduced proliferation (VX680, P < 0.05 for 0.1–10 μmol/L; ASC66, P < 0.05 for 1–10 μmol/L), striking inhibitory effects on tumorsphere formation were observed at even lower doses for ASC66 (P < 0.05, ASC66 at 0.1 μmol/L nonsignificant; Fig. 2D). The inhibitory profiles of tool compounds and validation by VX680 suggest that AURKs are necessary for self-renewal of TPCs in human GBMs.

Aurora kinase inhibition reduces the levels of activated histone H3, increases G2–M arrest, promotes polynucleation, and leads to apoptosis in TPC cultures

To confirm the ability of AURK inhibition to reduce proliferation of TPCs, we demonstrated that VX680 reduced (6969, P < 0.05 for 10 nmol/L dose; 6969/GS2, P < 0.001 for 100 nmol/L dose) phosphorylated (Ser-10) histone H3 levels in nestin-expressing 6969 and GS2 GBM cells (Fig. 3A–E). We found that all three human GBM cultures expressed AURKA-C, but not some other known targets of VX680 (Supplementary Fig. S2). Incubation of 6969 GBM cells with VX680 or the tool compound ASC58 caused G2–M arrest, polynucleation, and apoptosis (Fig. 3F–K). As predicted, ASC58 mimicked the cellular effects of VX680 only at higher doses (1 μmol/L). We confirmed that VX680 concentration dependently reduced phosphorylated histone H3 levels (10 nmol/L, P < 0.05; 100 nmol/L, P < 0.01) and induced polynucleation (effects mimicked by ASC58 treatment) in human primary GBM43 cells (Supplementary Fig. S3A–S3F). Our results demonstrate that both our synthesized kinase inhibitors expected to target AURKs and VX680 resulted in expected cellular effects,
including G2–M arrest, polynucleation, and apoptosis in human GBMs.

**Sequential therapy makes radioresistant TPCs vulnerable to AURK inhibition**

Depletion of tumor bulk cells induced cell cycling of the radioresistant TPC compartment 4 days following a single dose γ-irradiation (Fig. 1G). Therefore, we hypothesize that AURK inhibition for 3 days following a priming dose of radiotherapy will effectively block self-renewal capacity in GBM cultures (Fig. 4A). Proliferation of human primary GBM43 cells was reduced following γ-irradiation (10 Gy, \( P < 0.001 \)) and VX680 treatment (1 \( \mu \)mol/L, \( P < 0.001 \); Fig. 4). However, a priming dose of γ-irradiation followed by AURK inhibition shows no additional inhibition of proliferation compared with γ-irradiation alone (Fig. 4B). In contrast, γ-irradiation increased (\( P < 0.001 \)) and VX680 reduced (\( P < 0.01 \)) the fraction of self-renewing cells forming secondary tumorspheres (Fig. 4B).

VX680 treatment following a priming dose of radiotherapy almost completely abolished generated secondary tumorspheres (\( P < 0.001 \) vs. control or γ-irradiation, \( P < 0.05 \) vs. VX680 alone, Fig. 4). Reversal of the sequential treatment regimen (VX680 before γ-irradiation) showed a similar anti-proliferative effect, but less effective at targeting self-renewing TPCs (\( P < 0.01 \); Supplementary Fig. S4). Our data suggest that induction of cell cycling in TPCs following γ-irradiation optimizes the ability of the AURK inhibitor VX680 to target TPCs in cultured human GBMs.

**Sequential therapy reduces tumor growth in mice xenografted with human primary GBM cells**

To extend these results to an **in vivo** setting, human primary GBM43 cells expressing firefly luciferase (LUC) gene and EGFP were transplanted orthotopically into athymic nu/nu mice (Fig. 5A). One day following a single dose of γ-irradiation (5 Gy), proliferative bulk tumor cells were effectively targeted, and
the fraction of PDPN-expressing cells enriched ($P < 0.01$, Fig. 5B and C). To study the ability of VX680 to reduce tumor growth in intracranial xenografts, we confirmed that a single i.p. injection of VX680 (45–75 mg/kg/injection) reduced phosphorylated histone H3 at 48 hours after injection (Fig. 5D). We conclude that VX680 cross the blood–brain barrier (BBB) in our intracranial xenograft model.

To study whether radiotherapy and VX680 can cooperate to reduce tumor growth and prevent regrowth in xenografted mice, we divided mice ($n = 5$) into multiple cohorts (Fig. 5E). Isolation of EGFP$^+$ human GBM43 cells from treated mice demonstrated that radiotherapy induced apoptosis (Fig. 5F). Five consecutive and daily injections of VX680 (75 mg/kg/d) induced both G$_2$–M arrest and polynucleation. Interestingly, administration of VX680 after, but not before, the priming dose of $\gamma$-irradiation increased the fraction of apoptotic cells and reduced the fraction of proliferating cells compared with radiotherapy alone (Fig. 5F). Bioluminescence imaging showed that VX680 alone reduced tumor growth without affecting OS; an effect attributed to toxicity and increased body weight loss (Fig. 5G and H and Supplementary Fig. S5). For sequential treatment regimens, we observed that VX680 increased body weight loss (Fig. 5G and H and Supplementary Fig. S5). Five consecutive injections of VX680 after, but not before, $\gamma$-irradiation resulted in reduced body weight ($P < 0.05$) compared with $\gamma$-irradiation alone (Supplementary Fig. S5). Five consecutive injections of VX680 after, but not before, significantly reduced ($P < 0.01$) tumor growth and increased ($P < 0.05$) OS compared with $\gamma$-irradiation alone (Fig. 5G). In conclusion, our in vivo results suggest that induction of cell cycling of TPCs through a priming dose of radiotherapy effectively reduce tumor growth and extend OS.

Discussion

Preclinical findings show that a subpopulation of relatively dormant GBM cells is resistant to radiotherapy and temozolomide treatment (9, 11, 12). From a library of multitargeted kinase inhibitors, we confirmed previous findings demonstrating that AURK inhibition effectively reduces proliferation of cultured GBM cells (33, 34). Following a priming dose of ionizing radiation, we demonstrate that AURK inhibitors reduce the self-renewal capacity of TPCs in human GBM cultures. We show that a priming dose of ionizing radiation triggers cell cycling of TPCs in vivo, allowing AURK inhibition to induce apoptosis and polynucleation in xenografted GBM43 cells, an effect that was not observed using the AURK inhibitor VX680 alone. Importantly, reversed order of AURK inhibition preceding radiotherapy had no beneficial effect on tumor growth or survival of xenografted mice, suggesting that induced cell cycling of TPCs is a prerequisite for the efficacy of VX680. Administration of the AURKB/C inhibitor AZD1152-HQPA to mice intracranially grafted with the human GBM cell line U251 shows only a moderate survival benefit (33). The authors suggest the possibility that single-agent therapy might fail to target not actively cycling tumor cells and implicate a role
for combination therapy with radiotherapy and temozolomide treatment. Another study found that the AURK inhibitor ZM447439 enhanced the effects of radiotherapy in U251 GBM cells, further potentiated in the presence of temozolomide (35). Our study exemplifies the importance of sequential therapy regimens to target TPCs and non-TPC compartments in heterogeneous cancers. The failure of clinical trials to produce a survival benefit in patients with GBM is due to a multitude of factors, including incomplete surgical removal, off-target effects, poor BBB permeability, and genetic complexity of tumors. The emerging cancer stem cell concept emphasizes the need to better understand how intratumoral heterogeneity contribute to resistance to therapy, and to identify therapeutic targets on TPCs that are thought to underlie regrowth of GBMs and recurrence in patients. The thiazole antibiotic siomycin A was identified through a high-throughput compound screen for inhibitors of the transcription factor forkhead box M1b (FoxM1b; ref. 36). FOXM1 is a critical regulator of a number of genes enriched in multipotent NSCs, the maternal embryonic leucine zipper kinase (MELK) was found and later shown to be tightly associated with also overexpressed FOXM1 (41). On the basis of the multikinase inhibition profile of the tool compounds tested in our study, targeting of CDK2/5, AKT1, FGFR3, AURKB, and AURKC was predicted to most effectively reduce self-renewal of TPCs in cultured human GBMs (Fig. 2B, data not shown). Beyond the scope of our work, future studies should investigate whether inhibition of individual kinases or combinations effectively reduces self-renewal capacity for TPCs in human GBMs.

All three members of the AURK family play crucial roles at distinct stages of the mitotic phase. Therefore, AURK inhibitors represent an attractive approach to target proliferating GBM cells without affecting nondividing neurons and glial cells (42). The small-molecule pan-AURK inhibitor VX680 is known to suppress tumor growth in animal models (32, 43). In patients with GBM, expression of AURKA in tumors is associated with poor survival (44). In vitro, AURKA inhibition in GBM cell lines was cytotoxic and potentiated by ionizing radiation. Interestingly, RNAi of AURKA inhibited self-renewal capacity of TPCs in human primary GBM cultures and reduced tumorigenicity when cells were grafted in vivo (45).

As a potential therapeutic target in genetically distinct GBM subgroups, AURKB inhibition leads to perturbed cytokinesis, massive polyploidization, and cell death in both TP53-proficient and TP53-deficient cells (46, 47). AURKC is able to reconstitute AURKB function in AURKB-depleted cells (48), suggesting a redundant role during mitosis. AURKK is over-expressed in pediatric diffuse intrinsic pontine gliomas and high-grade gliomas (49). Incubation of pediatric glioma cell lines with VX680 reduced cell survival and resulted in polyploidy. Using GBM cell lines, the AURKB/C-specific inhibitor AZD1152 increased apoptosis and polyploidy in vivo, and impaired tumor growth in xenografted GBMs (33, 34). These studies and our results show that correct timing of AURK inhibitors represent a viable approach to target larger cohorts of patients with pediatric or adult glioma.

High interstitial fluid pressure in tumors and selectivity of the BBB against charged molecules contribute to the reduced access of small-molecule inhibitors into human GBMs. Interestingly, a markedly reduced effect of AZD1152-HQPA was demonstrated in intracranially grafted GBMs compared with subcutaneous tumors (33), suggesting limited bioavailability to the brain. We found that a single injection of VX680 was able to reduce Ser-10 phosphorylation of histone H3 and cause

Figure 4.
Radiotherapy-induced cell cycling of TPCs facilitates AURK inhibition and promotes apoptosis in human GBM. To study the effects of radiotherapy and AURK inhibition on proliferation and self-renewal in human GBM43 cells, we tested a sequential therapy regimen [10 Gy radiation followed by VX680 (1 μmol/L) treatment] compared with vehicle control, radiation, or VX680 (1 μmol/L) alone. A, radiotherapy and the sequential regimen (γ-irradiation plus VX680 treatment) effectively reduced proliferation compared with VX680-treated or control cultures. The fraction of radioresistant GBM43 cells efficiently showed increased secondary sphere-formation capacity following radiotherapy compared with control cultures. In contrast, VX680, and to a greater extent the sequential regimen, effectively reduced sphere-formation capacity of GBM43 cells compared with control cultures. The few remaining clusters following radiotherapy and VX680 treatment displayed reduced viability. B, quantification showed that γ-irradiation and the sequential regimen effectively reduced proliferation. A less pronounced effect was observed in VX680-only treated cells. The increased fraction of sphere-forming GBM43 cells following γ-irradiation was in stark contrast with the reduced sphere formation in cultures treated with VX680 or a sequential regimen (IR + VX680).
robust G2–M arrest in intracranial xenografts of human GBMs (Fig. 5D). As a possible explanation for the improved efficacy of VX680 in xenografted GBMs following ionizing radiation, it is possible that radiotherapy-induced vascular remodeling facilitated uptake of VX680 (Fig. 5G and H). Modification of the chemical structure and alternative delivery of AURK inhibitors are essential for future use against central nervous system tumors.

In conclusion, we anticipate that radiotherapy will remain an integral part of standard of care for patients with GBM. Instead of continuing extensive cycles of radiotherapy (that likely increase radioresistance of resistant TPCs), our study propose that multiple cycles of priming doses of ionizing radiation followed by targeted therapy against proliferating TPCs should inhibit TPC-induced regrowth and recurrence in patients with GBM. The concept of a priming regimen to trigger proliferation.

Figure 5. Sequential radiotherapy and AURK inhibition reduce tumor growth in human GBM xenografts. A, human GBM43 cells were lentivirally transduced with EGFP and the firefly luciferase (LUC) gene that allowed noninvasive measurements of tumor growth (bioluminescence), efficient isolation, and flow cytometry-mediated identification of tumor cells. B, to study the effect of radiotherapy on dormant and proliferating GBM cells as a function of PDPN expression, we performed radiotherapy, the following day performed i.p. injections of BrdUrd 2 hours before isolation of tumor cells, and then studied overlap of PDPN and BrdUrd using flow cytometry. C, a single dose of γ-irradiation (5 Gy) depleted proliferating tumor cells and markedly increased the fraction of EGFP–PDPN+ human GBM43 cells. D, the levels of Ser-10 phosphorylation were reduced 48 hours following a single i.p. injection of VX680 (45–75 mg/kg). E, to test the effects of radiotherapy and VX680 on tumor growth in mice xenografted with GBM43 cells, we divided groups of 5 mice into vehicle control, radiotherapy (IR, 5 Gy), VX680 (75 mg/kg/day × 5 days), radiotherapy plus VX680, and VX680 followed by radiotherapy. F, we labeled fixed cells with propidium iodide and performed flow-cytometry analysis to study how radiotherapy, VX680, or sequential regimens of radiotherapy and VX680 treatment regulated cell cycling, apoptosis, and polynucleation in tumor cells isolated from treated GBM43 xenografts. G, VX680 resulted in reduced tumor growth in mice xenografted with GBM43 cells compared with untreated (control) mice. Separate cohorts of mice xenografted with GBM43 cells received radiotherapy and two sequential regimens of radiotherapy and VX680 treatment. H, Kaplan-Meier curve showing survival of xenografted mice receiving combinations of γ-irradiation and VX680 treatment.
of TPCs has been studied in patients with leukemia (50). Our results show that better understanding of cell-cycling properties in interacting tumor cell compartments should be exploited to eradicate otherwise dormant and resistant TPCs in human GBM.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: N. Li, W.A. Weiss, A.I. Persson
Development of methodology: N. Li, D.J. Maly, J.L. Nakamura, C.D. James, K.M. Shokat, A.I. Persson
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N. Li, Y.H. Chanthery, D.W. Sirkis, A.I. Weiss, A.I. Persson
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N. Li, Y.H. Chanthery, D.W. Sirkis, W.A. Weiss, A.I. Persson
Writing, review, and/or revision of the manuscript: N. Li, J.L. Nakamura, M.S. Berger, W.A. Weiss, A.I. Persson
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N. Li, M.S. Berger, C.D. James, A.I. Persson
Study supervision: K.M. Shokat, W.A. Weiss, A.I. Persson

References


Radiotherapy Followed by Aurora Kinase Inhibition Targets Tumor-Propagating Cells in Human Glioblastoma

Nan Li, Dustin J. Maly, Yvan H. Chanthery, et al.

Mol Cancer Ther 2015;14:419-428. Published OnlineFirst December 18, 2014.

Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-14-0526

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2014/12/19/1535-7163.MCT-14-0526.DC1

Cited articles
This article cites 50 articles, 13 of which you can access for free at:
http://mct.aacrjournals.org/content/14/2/419.full#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
http://mct.aacrjournals.org/content/14/2/419.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://mct.aacrjournals.org/content/14/2/419.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.