Therapeutic Efficacy of Seliciclib in Combination with Ionizing Radiation for Human Nasopharyngeal Carcinoma

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Abstract

Purpose: Seliciclib is a small-molecule cyclin-dependent kinase inhibitor, which has been reported to induce apoptosis and cell cycle arrest in EBV-negative nasopharyngeal carcinoma cell lines. Because most nasopharyngeal carcinoma patients harbor EBV, we proceed to evaluate the cytotoxic effects of seliciclib in EBV-positive nasopharyngeal carcinoma models.

Experimental Design: Cytotoxicity of seliciclib was investigated in the EBV-positive cell line C666-1 and the C666-1 and C15 xenograft models. Caspase activities and cell cycle analyses were measured by flow cytometry. Efficacy of combined treatment of seliciclib with radiation therapy was also evaluated.

Results: Seliciclib caused significant cytotoxicity in the C666-1 cells in a time- and dose-dependent manner, with accumulation of cells in both sub-G1 and G2-M phases, indicative of apoptosis and cell cycle arrest, respectively. Caspase-2, -3, -8, and -9 activities were all increased, with caspase-3 being the most significantly activated at 48 h after treatment. These cells also showed a reduction of Mcl-1 mRNA and protein levels. Combined treatment of seliciclib with radiation therapy showed a synergistic interaction with enhanced cytotoxicity in C666-1 cells and delayed repair of double-strand DNA breaks. For in vivo models, significant delays in tumor growth were observed for both C666-1 and C15 tumors, which were associated with enhanced apoptosis as determined by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling and immunohistochemistry analyses.

Conclusions: Seliciclib enhanced the antitumor efficacy of radiation therapy in EBV-positive nasopharyngeal carcinoma, characterized by G2-M arrest, and apoptosis, associated with an induction in caspase activity. This process is mediated by reduction in Mcl-1 expression and by attenuation of double-strand DNA break repair.

Nasopharyngeal carcinoma is a unique epithelial malignancy, with distinct biological and epidemiologic features from other epithelial cancers of the head and neck region. Firstly, the EBV genome is present in >80% of nasopharyngeal carcinoma cases worldwide. Secondly, nasopharyngeal carcinoma is endemic in certain geographic regions such as in Southeast Asia, where the annual incidence is up to 1 in 2,000. The major curative treatment modality for nasopharyngeal carcinoma is ionizing radiation therapy (radiation therapy), achieving an overall 5-year survival rate of ~65% (1). Intensity-modulated radiation therapy with or without chemotherapy improves local control rates to ~100%, but unfortunately this is still associated with a significant 2-year distant failure rate of 40% (2). Hence, the need to develop novel therapies for nasopharyngeal carcinoma remains.

We have conducted several previous preclinical evaluations of novel therapeutic approaches, such as adenoviral gene therapy driving the expression of candidate genes from an EBV-specific promoter (3), and showed that nasopharyngeal carcinoma is particularly susceptible to proapoptotic therapies. Specifically, the therapeutic genes have included p53 (3), nFasL (4), E1A (5), and BimS (6). Because delivery of adenoviral-mediated gene therapy is limited to intratumoral injections, a systemically administered Bcl-2 antisense oligonucleotide was then evaluated, showing that, in combination with radiation therapy, this approach successfully caused regression of established nasopharyngeal tumors in vivo (7).

In the search for additional targets, global gene expression of primary human nasopharyngeal carcinoma samples showed...
alterations in cell cycle regulation (8). Cell proliferation is a tightly controlled process with the cyclin-dependent kinases (CDK) coordinating cell division to ensure that DNA synthesis and subsequent mitotic divisions are accurately executed (9). Deregulation of CDK activation, with associated loss of cell cycle control, is observed in almost all human malignancies. Consequently, inhibition of CDKs would appear to be a potentially fruitful target for cancer therapy, and several small-molecule inhibitors have been identified and evaluated (10).

One of the most promising compounds is seliciclib (R-roscovitine; CYC202), a small-molecule CDK inhibitor, which has been shown to have antiproliferative and antitumor effects in many human cancer models (11–14). This agent has now progressed to phase II clinical testing (15). Efficacy of seliciclib has been previously investigated in four human nasopharyngeal carcinoma cell lines (16), wherein apoptosis and cell cycle arrest were both observed. However, these were all EBV-negative models. Given that the vast majority of nasopharyngeal carcinoma patients harbor EBV-positive disease, it is important to evaluate the efficacy of this CDK inhibitor using more relevant EBV-positive nasopharyngeal carcinoma models and also to examine this agent in combination with radiation therapy, a curative modality in the management of nasopharyngeal carcinoma patients.

Materials and Methods

Cell line, reagents, and xenograft tumors

C666-1, an EBV-positive nasopharyngeal carcinoma cell line (17), was maintained in RPMI 1640 supplemented with 10% fetal bovine serum and 100 mg/L penicillin/streptomycin at 37°C, 5% CO₂. C15 xenograft tumors were passaged in vivo as described previously (18). Seliciclib was obtained from Cyclacel, dissolved in 100% DMSO, diluted as required into medium, and used as per recommendations from Cyclacel.

Viability assay for C666-1 cells treated with seliciclib with or without radiation therapy

The soluble tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS)] cell proliferation assay was used to evaluate cytotoxicity of seliciclib on C666-1 cells as described previously (7), because these cells cannot form colonies. Briefly, cells were seeded onto 96-well plates after passaging, with different concentrations of seliciclib added to the cells 24 h later. Irradiation was conducted at room temperature using a 157 Cs unit (Gammax Cell 40 Extractor; MDS Nordion) at a dose rate of 1.1 Gy/min. Cell viability was evaluated by adding 20 μL of the MTS reagent (Promega) to each well in a 96-well plate at the indicated time points, with absorbance measured at 492 nm with a SPECTRAFluor Plus fluorometer (Tecan) according to the manufacturer’s specifications.

Evaluation of expression of Mcl-1 mRNA and protein

Quantitative real-time PCR. C666-1 cells were treated with seliciclib at 30 μmol/L for 24, 48, or 72 h followed by RNA extraction using the RNeasy Mini Kit (Qiagen). Reverse transcription was done using SuperScript II Reverse Transcriptase (Invitrogen) according to the manufacturer’s recommendations. To measure Mcl-1 mRNA expression, quantitative real-time PCR was done using the ABI PRISM 7900 Sequence Detection System (Applied Biosystems) using the Mcl-1 forward 5′-GGTGCGCATCAGAATGT-3′ and reverse 5′-ATCAATGGGAGCCT-3′ primers. The relative fold change in RNA expression was calculated using the 2^-ΔΔCt method, where the average of ΔCt values for the amplicon of interest was normalized to that of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase and compared with that of control treated specimens.

Western blotting. The expression of Mcl-1 in C666-1 was investigated by Western blotting. Briefly, cells were washed with PBS and lysed with radioimmunoprecipitation assay buffer (Invitrogen) containing protease inhibitors. Twenty-six micrograms of extracted protein for each sample were separated on 10% polyacrylamide Tris-glycine gels (Invitrogen) and transferred to nitrocellulose membranes (Amerham Biosciences). Membranes were incubated at 4°C overnight with polyclonal antibodies to either anti-Mcl-1 (1:1,000; BD Biosciences) or glyceraldehyde-3-phosphate dehydrogenase (Santa Cruz Biotechnology). Blots were incubated with horseradish peroxidase conjugated to anti-rabbit antibody (1:3,000; GE Healthcare UK) and then analyzed with the enhanced chemiluminescence substrate system (Amerham Biosciences).

Quantitation of phosphorylated H2AX

C666-1 cells were treated with vehicle, or seliciclib (20 μmol/L), then exposed to 2 Gy radiation therapy. Cells were harvested at 15 min, 30 min, 60 min, 4 h, or 24 h post-radiation therapy. Staining for phosphorylated histone H2AX (+H2AX) was conducted as described previously (19). Briefly, cells were fixed with 3 mL of 70% ethanol, and kept at −20°C. After collecting all samples, cells were rehydrated with 1 mL cold TST buffer.
[20 mmol/L Tris-HCl (pH 8.0), 0.1% Tween 80, 100 mmol/L NaCl] on ice for 10 min and resuspended in 200 μl FITC-labeled mouse monoclonal anti-phosphorylated γ-H2AX antibody (1:500 dilution in TST; Upstate Biotechnology) for 2 h at room temperature. Cells were then washed twice in TBS and analyzed using the FACS Calibur.

Investigation of therapeutic efficacy of seliciclib with radiation therapy in nasopharyngeal xenograft tumors

All animal experiments used 6- to 8-week-old severe combined immunodeficient BALB/c female mice and were conducted in accordance with the guidelines of the Animal Care Committee, University Health Network. To generate C666-1 tumors, cells (10^7) were injected into the left gastrocnemius muscle of severe combined immunodeficient mice. Tumor growth was monitored by measuring tumor plus leg diameter as described previously (7). Treatments commenced once the tumor plus leg diameter reached 8.75 mm. Seliciclib (50 mg/kg/injection) was administered intraperitoneally twice per day on days 1 to 5 and 8 to 12. Two local radiation therapy treatments were delivered (6 Gy each) on days 2 and 9 using a 137Cs unit at a dose rate of 1.1 Gy/min. When radiation therapy was combined with seliciclib, radiation therapy followed the seliciclib injection within 2 h. The mice were monitored for tumor formation at least three times per week. Mice weight was also monitored as a global assessment of tolerance to these treatments. The same procedure was followed for the C15 xenograft tumor studies.

Assessment of apoptosis in xenograft tumors

After treatment for 12 days, mice were sacrificed 2 h post-seliciclib injection. The C666-1 tumors were excised and then immediately fixed in 10% formalin (in PBS) for 48 h, placed in 70% alcohol for 48 h, paraffin embedded, and sectioned (5 μm). The extent of terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was measured for the in situ detection of apoptotic cells using the In situ Death Detection kit (Roche Diagnostics).

Results

Cytotoxicity of seliciclib on EBV-positive C666-1 cells. Cytotoxicity of seliciclib (0, 10, 20, 30, 50, 75, and 100 μmol/L) on EBV-positive C666-1 cells was evaluated using the MTS assay conducted at 24, 48, 72, and 144 h after initiation of drug treatment. As shown in Fig. 1A, seliciclib caused a dose- and time-dependent cytotoxicity, with maximum reduction in viability seen following treatment with 100 μmol/L seliciclib for 144 h. The MTS data at this time point were selected to guide the subsequent experiments. Because C666-1 cells have a relatively long doubling time of ~30 h, the IC50 for these cells was determined to be 30 μmol/L.

Seliciclib induces caspase activity and G2-M arrest in C666-1 cells. To determine the mode of cytotoxicity of seliciclib on C666-1 cells, cell cycle profiles were investigated using flow cytometry. Compared with vehicle (DMSO)-treated cells, there was a dose- and time-dependent increase in the proportion of cells that had a DNA content corresponding to either sub-G_1 or G_2-M phases of the cell cycle, starting at 24 h after drug treatment (Fig. 1B). At 60 μmol/L seliciclib (2× IC50), the proportion of sub-G_1 cells reached 40% by 72 h post-drug exposure. This was accompanied by a decrease in the proportion of G_2-M cells, which was most likely due to the large number of apoptotic cells.

This apoptotic process was mediated via activation of caspase-2, -3, -8, and -9 (Fig. 1C), which was already apparent after 24 h drug exposure. C666-1 cells treated with 30 μmol/L (IC50) of seliciclib showed activation of all four caspases, with caspase-3 being the most significantly activated, to ~10-fold higher than the vehicle-treated cells.

Seliciclib induces apoptosis in vitro by suppressing Mcl-1 expression. In addition to inhibiting the cell cycle, the apoptotic effects of seliciclib have been described previously and found to be preceded by suppression of Mcl-1 (13), a member of the Bcl-2 family of antiapoptotic proteins. Indeed, Mcl-1 mRNA expression was suppressed as early as 24 h in the C666-1 cells treated with seliciclib 30 μmol/L (IC50) and continued to decline to ~20% of untreated cells by 72 h (Fig. 2A). Mcl-1 protein level was slightly reduced at 48 h, being further down regulated by 72 h (Fig. 2B), showing a good correlation between mRNA and protein expression levels.

Interaction of seliciclib with radiation. The effect of combining seliciclib treatment with radiation therapy was investigated in different sequences. The three treatment regimens included delivering drug 24 h before radiation therapy (S1-R2), combining drug with radiation therapy on the same day (R2-S2), or drug treatment administered 24 h post-radiation therapy (R1-S2). A lower concentration of seliciclib (20 μmol/L) was selected for this evaluation to avoid extensive cytotoxicity to allow detection of potential interactions. Similarly, C666-1 cells were treated with 4 Gy radiation therapy, which on its own reduced viability to 60% at 48 h (7). As observed in Fig. 3A, all three combination schedules were effective. However, the optimal combination that achieved the greatest level of toxicity was seliciclib first followed 24 h later by radiation therapy (4 Gy; S1-R2). This specific sequence of seliciclib followed by radiation therapy was additionally examined using two other radiation therapy doses of 2 Gy (Fig. 3B) or 6 Gy (Fig. 3C). The most significant “sensitization” was apparent with the 2 Gy radiation therapy combination, which reduced viability by 40% on day 3 and by >60% by day 5 (Fig. 3B) and was significantly more effective than radiation therapy alone at all time points examined. This compared favorably with 90% or 85% with the sequences of seliciclib and radiation therapy simultaneously (S2-R2) or seliciclib following radiation therapy (R1-S2), respectively.

The effects of seliciclib with radiation therapy on C666-1 cells were further analyzed using the Chou-Talalay combination index principle (20), showing that the interaction observed between both treatments was synergistic with the combination index <1 at all the tested combinations (Fig. 3D).

Seliciclib enhances radiation cytotoxicity by inducing caspase-mediated apoptosis and G_2-M arrest. The cell cycle effects of radiation therapy alone (2 Gy), seliciclib alone (20 μmol/L), or the combination of seliciclib followed by radiation therapy (24 h later) was similar to the previous data from seliciclib alone (Fig. 1B). All these treatments caused an increase in the proportion of cells in G_2-M starting at 24 h (Fig. 4A). The proportion of cells with a sub-G_1 DNA content did not change with radiation therapy alone at this dose but increased in cells treated with either seliciclib alone or the combination. The sub-G_1 population increased modestly from 8% at 24 h to 14% at 72 h in cells treated with both seliciclib and radiation therapy.

Caspase activation was further examined in C666-1 cells treated with radiation therapy alone or seliciclib followed by radiation therapy (Fig. 4B). Radiation therapy alone did not induce caspase activity, but the combined treatments of seliciclib followed by radiation therapy activated all four caspases, as early as 24 h, and reaching 4- to 5-fold activation. These fold
inductions are less than the previous caspase activation data (Fig. 1C), which were most likely due to the lower seliciclib dose used for the combination treatments (20 \( \mu \text{mol/L} \)) versus the IC_{50} dose (30 \( \mu \text{mol/L} \)) used in Fig. 1C. It may also due to the increase of cells in G2-M arrest and not undergoing apoptosis when exposed to combined treatment.

**Seliciclib enhances formation of double-strand DNA breaks in C666-1 cells.** Phosphorylation of \( \gamma \)-H2AX has been shown to be a sensitive indicator of DNA double-strand breaks produced by ionizing radiation and by drugs that cause double-strand breaks (19). In an effort to determine whether seliciclib could be augmenting radiation therapy toxicity via increasing DNA double-strand breaks in C666-1 cells, the proportion of cells staining with \( \gamma \)-H2AX was measured using flow cytometry (19). Figure 4C showed a higher percentage of \( \gamma \)-H2AX-expressing cells with the combination of seliciclib with radiation therapy, compared with radiation therapy alone, with the peak observed at 15 min post-radiation therapy (19.9% for seliciclib...
Seliciclib suppresses Mcl-1 expression in C666-1 cells. A, C666-1 cells were treated with vehicle alone (−) or seliciclib (+) at 30 μmol/L for 24, 48, or 72 h. RNA was then extracted for quantitative real-time PCR analysis. Mean ± SE relative expression from three independent experiments, each conducted in triplicate, compared with that of untreated cells (−). B, C666-1 cells were treated with vehicle alone (−) or 30 μmol/L seliciclib (+) for 24, 48, or 72 h. The resultant cell lysates were probed for Mcl-1 protein, with glyceraldehyde-3-phosphate dehydrogenase serving as the loading control.

Fig. 2. Seliciclib suppresses Mcl-1 expression in C666-1 cells. A, C666-1 cells were treated with vehicle alone (−) or seliciclib (+) at 30 μmol/L for 24, 48, or 72 h. RNA was then extracted for quantitative real-time PCR analysis. Mean ± SE relative expression from three independent experiments, each conducted in triplicate, compared with that of untreated cells (−). B, C666-1 cells were treated with vehicle alone (−) or 30 μmol/L seliciclib (+) for 24, 48, or 72 h. The resultant cell lysates were probed for Mcl-1 protein, with glyceraldehyde-3-phosphate dehydrogenase serving as the loading control.

and radiation therapy versus 16.7% for vehicle and radiation therapy; P = 0.05). In addition, the rate of repair of double-strand DNA breaks was decreased in the combination-treated cells with a slower loss of γ-H2AX signal over time, from 19.9% to 10.1% (50% reduction) at 24 h, compared with C666-1 cells treated with vehicle plus radiation therapy, from 16.7% to 3.8% (77% reduction).

In vivo evaluations of radiation therapy with seliciclib in nasopharyngeal carcinoma models. Finally, to evaluate the therapeutic efficacy of the combination of seliciclib and radiation therapy in vivo, two distinct EBV-positive nasopharyngeal carcinoma models were examined: C666-1 and C15 (17, 18). Seliciclib was administered intraperitoneally twice per day on days 1 to 5 and 8 to 12. Local tumor radiation therapy was administered on days 2 and 9 in this regimen. Vehicle- or seliciclib-treated mice had to be sacrificed for humane reasons between days 12 and 20 (Fig. 5A). In contrast, the radiation therapy-treated group showed tumor regression and survived until around day 57. The longest-surviving mice were those treated with the combination of seliciclib and radiation therapy, where the humane endpoint was not reached (maximum tumor plus leg diameter ~15 mm), for up to 80 days after initiation of treatment. This prolongation in survival is statistically significant (P < 0.05) and is particularly encouraging given that treatment was stopped on day 12.

Similar observations were obtained for the C15 tumors, wherein mice treated with seliciclib and radiation therapy had a significantly reduced tumor growth, surviving at least 56 days, which is >42 days longer than the control group (P < 0.05), with a maximum tumor diameter of ~10.5 mm. In contrast, mice treated with radiation therapy alone were sacrificed on day 52, once the tumor plus leg diameter reached 15 mm. Mouse body weight was also monitored, which remained unchanged during treatment. Once tumor burden increased significantly, mouse weight started to decline (data not shown).

The extent of treatment-induced apoptosis was assessed by immunohistochemical analysis (Fig. 5C). C666-1 tumors were removed 2 h after the last treatment (day 13), fixed, and embedded in paraffin. Seliciclib treatment alone increased the proportion of TUNEL-positive tumor cells (12.5%), similar to that observed for the radiation therapy-treated tumors (17.5%). However, the combination of seliciclib with radiation therapy caused the most significant increase in TUNEL-positive tumor cells to 40.8% (P = 0.01).

Discussion

This is the first report showing therapeutic efficacy of seliciclib combined with radiation therapy in two EBV-positive nasopharyngeal carcinoma models. Seliciclib is a 2,6,9-trisubstituted purine analogue, which competes with ATP for the catalytic binding site on CDKs (21), with demonstrated antitumor activity in many human cancer models, including myeloma (12), chronic lymphocytic leukemia (22), Ewing’s sarcoma (14), and breast carcinoma (11). One major mechanism of action involves the inhibition of CDK2/cyclin E activity, leading to cell cycle arrest (23). Seliciclib also interacts with CDK7/cyclin H and CDK9/cyclin T to inhibit RNA polymerase II-dependent transcription by blocking phosphorylation of its COOH-terminal domain (23, 24), thereby reducing expression of several cyclins including cyclins D1, A, and B1. In turn affects retinoblastoma phosphorylation (23, 24), leading to accumulation of cells in G2-M, at the expense of a reduced population of cells in the G1 and S phases of the cell cycle (23, 24).

Seliciclib also induces apoptosis via inhibition of the transcription of antiapoptotic genes such as Mcl-1, survivin, or X-linked inhibitor of apoptosis (23, 24). These pleiotropic cellular and biochemical effects are corroborated by our current data in nasopharyngeal carcinoma, wherein seliciclib caused significant cytotoxicity in the EBV-positive nasopharyngeal carcinoma C666-1 cells in both a time- and dose-dependent manner, with accumulation of treated cells in both sub-G1 and G2-M, indicative of apoptosis and cell cycle arrest, respectively. Activity of caspase-2, -8, and -9 were all induced by seliciclib alone, suggesting that both death receptor- and mitochondria-mediated apoptosis were occurring, with maximal increase in the activity of the convergent caspase-3 (Fig. 1C) at 48 h.

The mechanism of seliciclib-induced apoptosis in C666-1 cells was further examined by evaluating Mcl-1, a key antiapoptotic member of the Bcl-2 family. As previously reported for myeloma and other systems (23, 24), seliciclib-treated cells showed reduced Mcl-1 at both transcriptional and translational levels, with decrease in Mcl-1 mRNA observed as early as 24 h followed by Mcl-1 protein reduction (Fig. 2A and B). These data are consistent with the time course of apoptosis observed for the C666-1 cells (Fig. 1B and C).
To date, there has been only one previous study of seliciclib in nasopharyngeal carcinoma, showing a similar induction of apoptosis and cell cycle arrest in EBV-negative cell lines with IC₅₀ values ranging from 13.8 to 22.1 μmol/L. Surprisingly, up-regulation of Bcl-2 was observed at 12 to 24 h, which became down-regulated after 72 h exposure. Given that >80% of nasopharyngeal carcinoma worldwide are EBV-positive (7), the more relevant EBV-positive model was examined, showing a slightly higher IC₅₀ in C666-1 cells at 30 μmol/L (Fig. 1A). Bcl-2 levels were not measured in the current study because previous interventions have not shown any significant changes (25), and its expression level provided no prognostic value in our clinical-molecular evaluations (26).

Given that radiation therapy is the sole modality with a curative role in nasopharyngeal carcinoma, seliciclib was examined in combination with radiation therapy. The potential advantage of this combination was also supported by previous reports on seliciclib inhibiting DNA damage repair when combined with chemotherapy or radiation therapy (11, 27, 28). The current evaluation of seliciclib combined with radiation therapy showed a synergistic interaction with enhanced cytotoxicity in C666-1 cells (Fig. 3D). The proportion of cells undergoing apoptosis was significantly higher for the combination treatment, compared with radiation therapy alone, and was associated with greater induction of caspase activation (Fig. 4A and B).

In this tumor model, seliciclib also inhibited the repair of DNA double-strand breaks, shown by the higher signal of γ-H2AX in C666-1 cells treated with seliciclib and radiation therapy, along with a slight delay in repair kinetics (Fig. 4C). Similar results had been reported in a recent study, which detected radiosensitivity of seliciclib on a lung cancer cell line, by increasing apoptosis and caspase-3 activity as well as impairing the DNA repair process (29). The mechanisms underlying this interaction might relate to seliciclib-induced inhibition of RNA polymerase II activation leading to interference with the transcriptional-coupled component of DNA repair (11, 27, 28). This beneficial interaction also appears to be independent of p53, given the enhanced interaction between seliciclib and radiation therapy described in mp53 breast cancer models, which was also associated with increased DNA breaks (11). Taken together, these results clearly show that seliciclib significantly enhanced radiation therapy response in human cancer models potentially through interference with the repair of double-strand DNA breaks.

The in vivo effects of systemically administered seliciclib combined with local tumor radiation therapy was very impressive, shown in two distinct EBV-positive nasopharyngeal carcinoma models (Fig. 5A and B). Significant delays in tumor growth were observed for both tumors, translating to a prolongation in survival. This was associated with significantly...
enhanced apoptosis (or necrosis) in tumors treated with this combination as documented by TUNEL analysis (Fig. 5C). Reduction of Mcl-1 protein expression was also observed in the xenograft tumors as evaluated via immunostaining (data not shown). The treated mice also maintained their body weight (data not shown), and the combined treatments appear to be well tolerated. These data corroborate the only previous in vivo evaluations of radiation therapy combined with seliciclib, although a much higher radiation therapy dose of 7.5 Gy was used, but in vivo apoptosis was not evaluated in these studies (11, 28).

The striking in vivo data would support the evaluation of this combination in human clinical trials. Seliciclib has indeed been evaluated in eight patients with locally advanced nasopharyngeal carcinoma, reporting >25% reduction in clinically measured cervical lymph nodes in three of eight evaluable patients, with minimal side effects (16) Hence, efficacy of this agent, particularly in combination with radiation therapy, definitely warrants future evaluations in the context of phase II clinical trials for patients with nasopharyngeal carcinoma. Currently, patients with locally advanced nasopharyngeal carcinoma are treated with radiation therapy combined with...
chemotherapy such as cisplatin, which unfortunately is associated with significant long-term normal tissue toxicities, particularly hearing loss (30, 31); hence, any novel therapeutic modalities that could enhance the long-term cure rate of radiation therapy for nasopharyngeal carcinoma would be welcomed.

In conclusion, seliciclib enhanced antitumor efficacy of radiation therapy in EBV-positive nasopharyngeal carcinoma, characterized by G2-M arrest, and apoptosis, associated with induction of caspase activity. This process is mediated by reduction of Mcl-1 expression and the interference in the repair of double-strand DNA breaks. Given the impressive in vivo data, the combination of seliciclib and radiation therapy should be assessed in a phase II clinical trial for patients with nasopharyngeal carcinoma.

Disclosure of Potential Conflicts of Interest

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References


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