Bortezomib for Glioblastoma

Bortezomib is a boronic acid-based potent proteasome inhibitor that has been actively studied for its anti-tumour effects through inhibition of the proteasome. The proteasome is a key component of the ubiquitin-proteasome pathway that is critical for protein homeostasis, regulation of cellular growth, and apoptosis. Overexpression of polo-like kinase 4 (PLK4) is commonly reported in tumour cells and increases their invasive and metastatic abilities. In this study, we established a cell model of PLK4 knockdown and overexpression in LN-18, A172 and LN-229 cells and found that knockdown of PLK4 expression enhanced the anti-tumour effect of bortezomib. We further found that this effect may be mediated by the PTEN/PI3K/AKT/mTOR signalling pathway and that the apoptotic and oxidative stress processes were activated, while the expression of matrix metalloproteinases (MMPs) was down-regulated. Similar phenomenon was observed using in vitro experiments. Thus, we speculate that PLK4 inhibition may be a new therapeutic strategy for GBM [1)].

The addition of bortezomib to current standard radiochemotherapy in newly diagnosed glioblastoma patients was tolerable. The PFS and OS rates appeared promising, with more benefit to MGMT methylated patients. Further clinical investigation is warranted in a larger cohort of patients [2)].

Jane et al. investigated the sensitivity of a panel of glioma cell lines (U87, T98G, U373, A172, LN18, LN229, LNZ308, and LNZ428) to TRAIL alone and in combination with the proteasome inhibitor bortezomib. Analysis of these cell lines revealed marked differences in their sensitivity to these treatments, with two (LNZ308 and U373) of the eight cell lines revealing no significant induction of cell death in response to TRAIL alone. No correlation was found between sensitivity of cells to TRAIL and expression of TRAIL receptors DR4, DR5, and decoy receptor DcR1, caspase 8, apoptosis inhibitory proteins XIAP, survivin, Mcl-1, Bcl-2, Bcl-Xl, and cFLIP. However, TRAIL-resistant cell lines exhibited a high level of basal NF-κB activity. Bortezomib was capable of potentiating TRAIL-induced apoptosis in TRAIL-resistant cells in a caspase-dependent fashion. Bortezomib abolished p65/NF-κB DNA-binding activity, supporting the hypothesis that inhibition of the NF-κB pathway is critical for the enhancement of TRAIL sensitization in glioma cells. Moreover, knockdown of p65/NF-κB by shRNA also enhanced TRAIL-induced apoptosis, indicating that p65/NF-κB may be important in mediating TRAIL sensitivity and the effect of bortezomib in promoting TRAIL sensitization and apoptosis induction [3)].

Premkumar et al. demonstrated that proteasome inhibitors, such as bortezomib, dramatically sensitized highly resistant glioma cells to apoptosis induction, suggesting that proteasomal inhibition may be a promising combination strategy for glioma therapeutics.

They examined whether bortezomib could enhance response to HDAC inhibition in glioma cells. Although primary cells from glioblastoma multiforme (GBM) patients and established glioma cell lines did not show significant induction of apoptosis with vorinostat treatment alone, the combination of vorinostat plus bortezomib significantly enhanced apoptosis. The enhanced efficacy was due to proapoptotic mitochondrial injury and increased generation of reactive oxygen species. Our results also revealed that combination of bortezomib with vorinostat enhanced apoptosis by increasing Mcl-1 cleavage, Noxa upregulation, Bak and Bax activation, and cytochrome c release. Further

downregulation of Mcl-1 using shRNA enhanced cell killing by the bortezomib/vorinostat combination. Vorinostat induced a rapid and sustained phosphorylation of histone H2AX in primary GBM and T98G cells, and this effect was significantly enhanced by co-administration of bortezomib. Vorinostat/bortezomib combination also induced Rad51 downregulation, which plays an important role in the synergistic enhancement of DNA damage and apoptosis. The significantly enhanced antitumor activity that results from the combination of bortezomib and HDACIs offers promise as a novel treatment for glioma patients [4].

One resistance mechanism in malignant gliomas (MG) involves nuclear factor-κB (NF-κB) activation. Bortezomib prevents proteasomal degradation of NF-κB inhibitor α (NFKBIA), an endogenous regulator of NF-κB signaling, thereby limiting the effects of NF-κB on tumor survival and resistance. A presurgical phase II trial of bortezomib in recurrent MG was performed to determine drug concentration in tumor tissue and its effects on NFKBIA. Patients were enrolled after signing an IRB-approved informed consent. Treatment was bortezomib 1.7 mg/m(2) IV on days 1, 4, and 8 and then surgery on day 8 or 9. Post-operatively, treatment was Temozolomide (TMZ) 75 mg/m(2) PO on days 1-7 and 14-21 and bortezomib 1.7 mg/m(2) on days 7 and 21 [1 cycle was (1) month]. Ten patients were enrolled (8 M and 2 F) with 9 having surgery. Median age and KPS were 50 (42-64) and 90 % (70-100). The median cycle post-operatively was 2 (0-4). The trial was stopped as no patient had a PFS-6. All patients are deceased. Paired plasma and tumor bortezomib concentration measurements revealed higher drug concentrations in tumors than in plasma; NFKBIA protein levels were similar in drug-treated vs. drug-naïve tumor specimens. Nuclear 20S proteasome was less in postoperative samples. Postoperative treatment with TMZ and bortezomib did not show clinical activity. Bortezomib appears to sequester in tumor but pharmacological effects on NFKBIA were not seen, possibly obscured due to downregulation of NFKBIA during tumor progression. Changes in nuclear 20S could be markers of the bortezomib effect on tumor [5].

McCracken et al. conducted a phase I trial of dose-escalating temozolomide with bevacizumab and the proteasome inhibitor bortezomib for patients with recurrent disease. Three groups of three patients were scheduled to receive daily doses of temozolomide at 25, 50, and 75 mg/m2. Fixed doses of bortezomib and bevacizumab were given at standard intervals. Patients were monitored for dose-limiting toxicities (DLT) to determine the maximum-tolerated dose (MTD) of temozolomide with this regimen. No DLT were seen in the first two groups (25 and 50 mg/m2 temozolomide). One patient in the 75 mg/m2 group experienced a grade 4 elevation of ALT and three more patients were accrued for a total of six patients at that dose level. No other DLT occurred, thus making 75 mg/m2 the MTD. Progression-free survival was 3.27 months for all patients and mean overall survival was 20.75 months. The MTD of temozolomide was 75 mg/m2 in combination with bevacizumab and bortezomib for recurrent glioblastoma. Only one patient experienced a severe (Grade 4) elevation of ALT. This study will provide the framework for further studies to elicit effectiveness and better determine a safety profile for this drug combination [6].

Oncolytic viruses, proteasome inhibitors, and natural killer (NK)-cell immunotherapy have all been studied extensively as monotherapies but have never been evaluated in combination. Synergetic treatment of oncolytic virus-infected glioblastomas with a proteasome inhibitor induces necroptotic cell death to enhance NK-cell immunotherapy, prolonging survival against human glioblastoma [7].

The proteasome inhibitor bortezomib is effective for a variety of tumors, but not for GBM. The authors' goal was to demonstrate that bortezomib can be effective in the orthotopic GBM murine model if the appropriate method of drug delivery is used. In this study the Alzet mini-osmotic pump was used to bring the drug directly to the tumor in the brain, circumventing the blood-brain barrier; thus making bortezomib an effective treatment for GBM. METHODS The 2 human glioma cell lines, U87 and U251, were labeled with luciferase and used in the subcutaneous and intracranial in vivo tumor models. Glioma cells were implanted subcutaneously into the right flank, or intracranially into the frontal cortex of athymic nude mice. Mice bearing intracranial glioma tumors were implanted with an Alzet mini-osmotic pump containing different doses of bortezomib. The Alzet pumps were introduced directly into the tumor bed in the brain. Survival was documented for mice with intracranial tumors. RESULTS Glioma cells were sensitive to bortezomib at nanomolar quantities in vitro. In the subcutaneous in vivo xenograft tumor model, bortezomib given intravenously was effective in reducing tumor progression. However, in the intracranial glioma model, bortezomib given systemically did not affect survival. By sharp contrast, animals treated with bortezomib intracranially at the tumor site exhibited significantly increased survival. CONCLUSIONS Bypassing the blood-brain barrier by using the osmotic pump resulted in an increase in the efficacy of bortezomib for the treatment of intracranial tumors. Thus, the intratumoral administration of bortezomib into the cranial cavity is an effective approach for glioma therapy [8].

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