Marizomib (NPI-0052) Activity as a Single Agent in Malignant Glioma
Kaijun Di1, Xing Gong2, Dana M. Curticiu3, Michael Palladino4, and *Daniela A. Bota1,2,5

(1) Department of Neurological Surgery, UC Irvine School of Medicine; (2) Department of Neurology, UC Irvine School of Medicine; (3) Department of Plant Breeding and Genetics, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania; (4) Triphase Accelerator U.S. Corporation, San Diego, CA; (5) Chao Family Comprehensive Cancer Center, UC Irvine, USA

INTRODUCTION

Glioblastoma multiforme (GBM) is a very aggressive tumor and highly resistant to conventional chemotherapy. The current standard of care involves maximizing local tumor control by using extensive DNA damage and disrupting the mitotic machinery of the cells, and averted not only the malignant cells but also the normal neural tissues, generating long-standing neurotoxicity in cancer survivors.1

The ubiquitin proteasome pathway is a key regulator in maintaining cellular homeostasis. It is responsible for the degradation of intracellular proteins including not only denatured, misfolded, and aged proteins, but those that regulate critical signaling pathways. Defects within these pathways are associated with a number of diseases, including cancer. Pre-clinical studies have demonstrated that malignant cells are more susceptible to the cytostatic effects of proteasome inhibition than normal cells, leading to the development of a novel anti-cancer strategy by targeting proteasome. Bortezomib (PS-341, Velcade®) is the first proteasome inhibitor whose bicyclic -lactone -lactam structure differs significantly from other peptide based proteasome inhibitors such as bortezomib and carfilzomib. Marizomib has a broader inhibition profile for the 20S proteasome. Marizomib is an irreversible proteasome inhibitor whose bicyclic -lactone -lactam structure differs significantly from other peptide based proteasome inhibitors such as bortezomib and carfilzomib. Marizomib has a broader inhibition profile for the 20S proteasome. Marizomib has also been shown to inhibit the chymotrypsin-like (CT-L), caspase-like (CL-L), and trypsin-like (T-L) activities of the 20S proteasome. Marizomib has also been used to activate a variety of caspases i.e., 3, 8, and 9, secondary to the buildup of ROS (reactive oxygen species) and activated proteases in the cells affected by proteasome activity, and thus to induce apoptotic cell death.2

RESULTS

METHODS

Drug: Marizomib (NPI-0052) was provided by Michael Palladino (Triphase Accelerator U.S. Corporation).

Cell lines: The primary brain tumor stem-like cells (GSCs) (low-grade glioma DB29, DB30, high-grade glioma DB17, DB26, DB28, DB30, meningioma-SC-M1, meningioma-SC-D1, and neural stem cell progenitors (NSC-SC27, NSC-SC93, and DB31) were isolated from patients as previously described. All the protocols were approved by the Institutional Review Board at University of California Irvine and Children’s Hospital of Orange County. The established malignant human glioma cell lines, U-251 MG and D-54 MG, were gifts from Dr. Daniel Sgouros at Duke University. The culture condition was same as previously described.3

20S proteasome activity assay: The proteasomal chymotrypsin-like (CT-L) activity was measured using the Proteasome Activity Assay Kit recommended by the manufacturer (Chemicon International Inc.).

Wound closure assay: Cells were grown to full confluence. Similar sized wounds were then induced to monolayer cells by scraping a gap using a micropipette tip. The time required for ‘wound closure’ was monitored and photographed by inverted microscope.

Antibodies used were Cleaved Caspase-3 (aspartic acid residue) cleaved PARP, and actin. Actin was the internal control.

Biotinylated Cell Adhesion Assay:

Figure 1. Marizomib inhibits proteasome activity in glioma cells.

Figure 2. Malignant GSCs and established glioma cell lines are more sensitive to marizomib-induced proteasome inhibition than NSCs, low-grade GSCs and primary meningioma cultures.

Figure 3. Marizomib inhibits the motility of glioma cells.

Figure 4. Marizomib increases apoptosis and Caspase-3 activation in glioma cells.

Figure 5. Marizomib increases ROS generation in glioma cells.

Figure 6. NAC pretreatment quenches ROS induction, and increases glioma cell survival upon marizomib treatment.

Figure 7. NAC blocks Caspase-3 activation induced by marizomib.

REFERENCES


CONCLUSION

Marizomib is an effective in-vitro agent, able to inhibit cell proliferation and migration in malignant glioma cells.

Marizomib activates Caspase-3 and causes apoptotic cell death.

A major mechanism of action for marizomib-induced apoptosis in glioma cells is ROS generation, which could be blocked by NAC pretreatment.

Marizomib selectively kills high-grade GSCs and that the NSCs are relatively resistant to this drug, indicating that marizomib might be able to offer malignant glioma tumor control with limited neurotoxicity.