The Novel Phospholipid Ether Analog CLR1404 Decreases Glioblastoma Stem Cell Proliferation, Suppresses GBM Growth, and Improves Survival

Paul A. Clark1, Hong-En Chen1, Mohamed Mohamed1, Irawati K. Kandela1, Marc A. Longino1,2,3, Anatoly Pinchuk1, James P. Weichert1,4, John S. Kuo1,2,4

Departments of 1Neurological Surgery, 2Human Oncology, and 3Radiology, and 4Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI, 53792-8660, USA; and Rharma Biotech, Inc., Hayward, CA 94545, USA

Introduction
Cancer Stem Cell Hypothesis

CSCs exhibit both self-renewal and multipotential differentiation, and a cancer stem cell paradigm has been advanced to explain the development and recurrence of cancer. Several CSC markers have been identified, including CD133, CD44, and ALDH1. However, no single antibody is uniformly positive for all CSCs. Therefore, a functional assay is required to enrich for CSCs. The optimal CSC assay involves orthotopic tumor initiation in NOD-SCID mice. CLR1404 and analogs were obtained from Novelos Therapeutics (Madison, WI). Proliferation assays were performed by addition of CLR1404 to GBM cell cultures. GSCs were treated with CLR1404, dosed to single cells, plated at 50-1000 cells in a 96-well plate, and allowed to form spheres (≤2 weeks). CLR1404 inhibition of the AKT signaling pathway in GSCs was assessed using immunoblot analysis. In vivo, GSCs were pre-treated for 24 hours with CLR1404 analogs prior to orthotopic implantation of 20,000 live cells into immunodeficient mice. Survival curves were then constructed.

Results and Conclusion: CLR1404 anti-proliferative effects were seen on all 7 different GSC and GBM lines tested with IC50 values ranging between 5-10 µM using MTS assay; control phospholipid ether CLR1404 and its analogs exhibit cancer cell-specific uptake and prolonged retention in 57/61 cancer cell lines and xenografts (including GBM) due to affinity for cancer stem cell (GSC) sub-population exhibiting therapeutic resistance and hypothesized to drive tumor recurrence. Therefore, GSC-specific targeting is likely critical for improving outcome. The orthotopic tumor initiation in NOD-SCID mice. CLR1404 and analogs were obtained from Novelos Therapeutics (Madison, WI). Proliferation assays were performed by addition of CLR1404 to GBM cell cultures. GSCs were treated with CLR1404, dosed to single cells, plated at 50-1000 cells in a 96-well plate, and allowed to form spheres (≤2 weeks). CLR1404 inhibition of the AKT signaling pathway in GSCs was assessed using immunoblot analysis. In vivo, GSCs were pre-treated for 24 hours with CLR1404 analogs prior to orthotopic implantation of 20,000 live cells into immunodeficient mice. Survival curves were then constructed.

Methods: Multiple sphere-forming GSC lines were isolated from patient specimens with IRB approval, and rigorously validated for self-renewal, multi-lineage potential, and high efficiency of tumor initiation in NOD-SCID mice. CLR1404 and analogs were obtained from Novelos Therapeutics (Madison, WI). Proliferation assays were performed by addition of CLR1404 to GBM cell cultures. GSCs were treated with CLR1404, dosed to single cells, plated at 50-1000 cells in a 96-well plate, and allowed to form spheres (≤2 weeks). CLR1404 inhibition of the AKT signaling pathway in GSCs was assessed using immunoblot analysis. In vivo, GSCs were pre-treated for 24 hours with CLR1404 analogs prior to orthotopic implantation of 20,000 live cells into immunodeficient mice. Survival curves were then constructed.

Conclusions and Future Directions
- The novel phospholipid ether CLR1404 and its analogs specifically label and are retained long-term in both glioblastoma multiforme (GBM) and GBM cancer stem cells (GSCs).
- CLR1404 analogs inhibit GSC and GBM cell proliferation in vitro and in vivo, and suppress GBM growth and improve survival in orthotopic mouse models.
- CLR1404 analogs at least partially exert therapeutic benefit via inhibition of AKT activation, a major molecular hub for oncogenic growth and survival. Analysis of other potential molecular mechanisms is currently underway.
- CLR1404’s therapeutic potential against GBM and its GSCs, combined with previously demonstrated tumor cell targeting specificity of CLR1404 and its analogs, provides strong evidence for the potential of novel CLR1404-based therapies to improve GBM outcomes.

Acknowledgements

References