Development of Novel Imidazotetrazine Prodrugs for the Treatment of Glioblastoma

Riley L. Svec and Paul J. Hergenrother

Glioblastoma (GBM) is the most prevalent, infiltrative, and lethal primary brain tumor, with only 10% of patients surviving five years. Despite persistent attempts to treat patients with state-of-the-art therapies including immunotherapy, the current standard-of-care for GBM remains surgical debulking followed by radiation and temozolomide (TMZ), a small molecule DNA alkylating agent. Although TMZ was first synthesized >30 years ago and received FDA-approval for primary GBMs in 2005, it remains the only approved imidazotetrazine anticancer drug. This likely stems from synthetic challenges associated with the sensitivity of the tetrazine and its accompanying intermediates, which prohibit conventional medicinal chemistry approaches.

TMZ is a prodrug activated in aqueous solutions ($t_{1/2}$ ~ 2h *in vitro* and *in vivo*) that ultimately releases methyldiazonium, the active alkylating component. Although the mechanism of hydrolysis has been well-established, there is little information on the relationship between prodrug half-life and anticancer activity. As such, novel synthetic methods were developed that provided access to a diverse panel of C8-substituted imidazotetrazines. Comparison of the activity against GBM cells in culture and electronic substituent effects revealed a direct relationship between the group at C8 and the aqueous stability of the prodrug, enabling the prediction of compounds that would display suitable half-lives *in vitro* and *in vivo*.

This discovery allowed for the rational design of compounds that not only demonstrated appropriate half-lives, but also possessed additional advantageous properties such as increased blood-brain barrier (BBB) penetration. Augmented BBB-permeability relative to TMZ was identified *in vivo* for a number of compounds, which directly led to increased efficacy head-to-head versus TMZ in aggressive orthotopic mouse models of GBM. In addition to increasing efficacy, the dramatic partitioning to the brain alleviated drug-induced hematological toxicity (dose-limiting for TMZ) *in vivo*. The relationships elucidated herein led to the advancement of several compounds that outperformed TMZ in critical preclinical studies.