In vivo imaging of immunotherapeutic glioma model

Glioblastoma remains one of the deadliest diseases faced by patients, with a median survival of less than 15 months. Therapeutic advancements in the field rely heavily on animal models, and the use of human brain tumor cells in mice has been a mainstay in neuro-oncology research for decades, particularly in the pre-clinical testing of new agents prior to entry into human clinical trials. Although these animal models implanted with human tumor cells (commonly referred to as xenograft models) produce many features characteristic of the human disease, one fundamental limitation remains: the use of animals lacking an intact immune-system (i.e. immunodeficient). The lack of an intact host immune-response undoubtedly alters tumor growth and behavior in mice. Furthermore, as immunotherapy, the use of a patient's own immune system to combat illnesses, gains popularity as an approach to treating many cancers, the use of mice models with an intact immune system (i.e. immunocompetent) becomes increasingly more valuable. As such, the Glioma-261 (GL261) model has gained traction as an excellent mouse glioma model. When implanted into the brain of an immunocompetent mouse, GL261-derived tumors express many of the features typical of human glioblastoma, and do not require the use of immunodeficient mice.

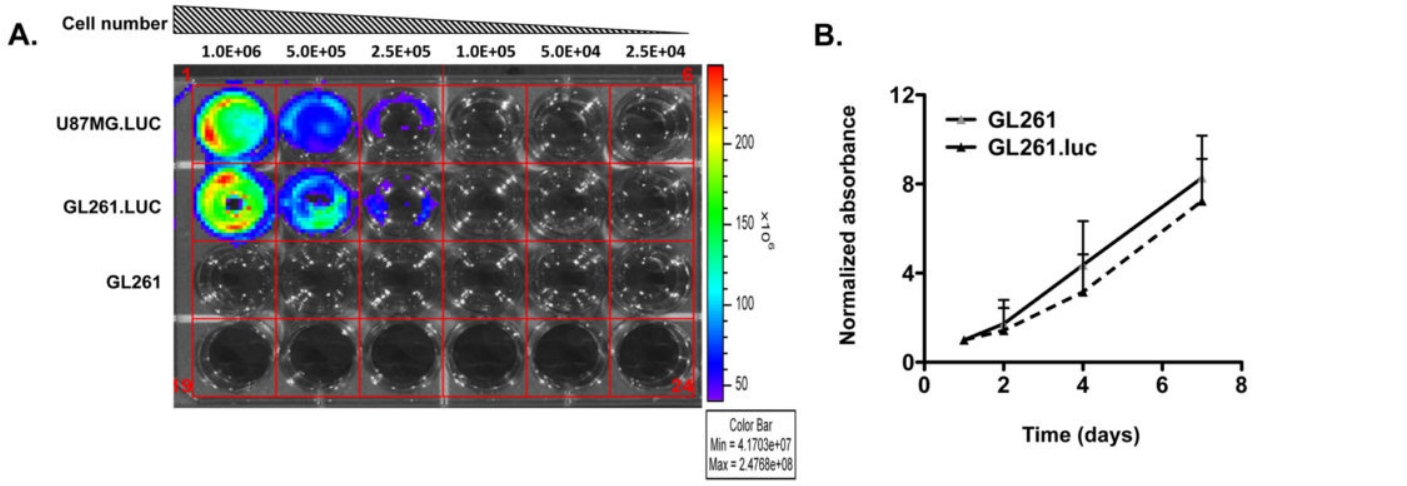

Fig.1. (A) U87.luc, GL261.luc and GL261 (negative control) cells were plated at densities of 1.0E+06, 5.0E+05, 2.5E+05, 1.0E+05, 5.0E+04, and 2.5E+04 cells/well from left to right. Luminescence (photon/sec/sr/cm2) was measured by the IVIS Lumina imaging station. (B) GL261.luc cells do not cause a difference in proliferation, as demonstrated by the CellTiter 96 AQueous One Solution Cell Proliferation Assay. The graph shows fold increase, relative to day 1, as determined by comparing the average absorbance value (mean ± SEM) at the specified time point to the average value at day 1 (unpaired t-test values for comparisons between each cell lines: P = 0.7796).

A non-invasive tumor imaging modality remains largely elusive, and real time measurement of tumor growth would greatly improve the ability to study therapeutic interventions. Therefore, we have developed a method relying on bioluminescence imaging (BLI), which involves the detection of photons emitted as a result of energy-dependent reactions catalyzed by cells or organisms that have been genetically-modified to express an enzyme such as luciferase. The reaction involves luciferase catalyzing the oxidation of luciferin, a cost-efficient substrate that can be safely injected into mice, and the resulting oxidation produces light that can be detected without sacrificing the mice. Genetic modification of the GL261 cell lines was achieved by way of transfection with a viral vector containing firefly luciferase (Fluc). Reproducible, serial imaging was achieved with a commercially available imaging system: the Xenogen IVIS Lumina system. Lastly, we demonstrated that Fluc transfection does not alter the viability of the GL261 cells, as proliferation studies performed both in a cell culture setting (Fig. 1) and in a mouse model (Fig. 2) noted no significant differences in growth patterns.

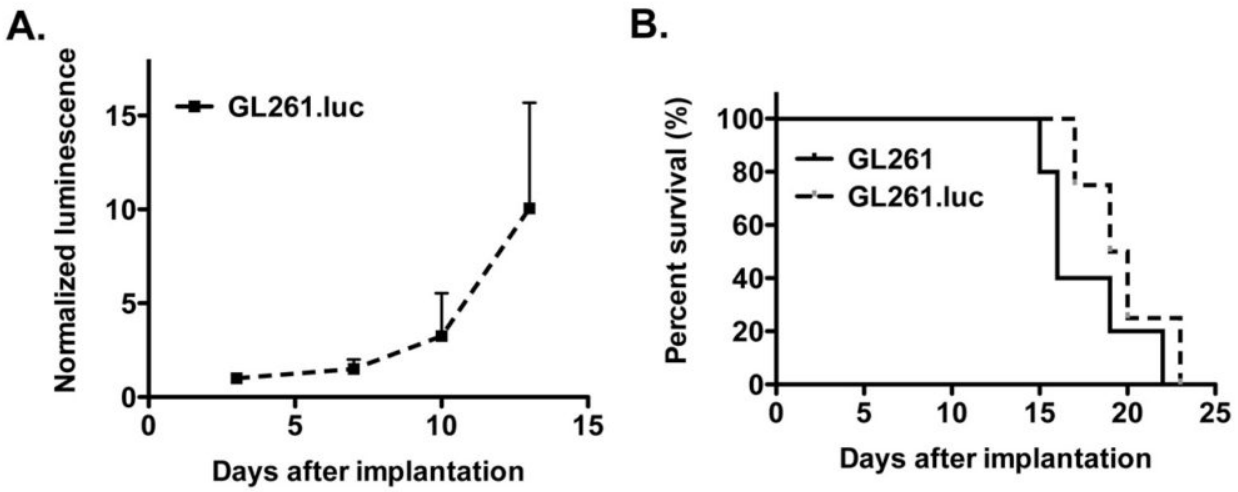

Fig. 2. (A) Bioluminescence monitoring demonstrates progressive growth of intracranial GL261.luc tumor in C57BL/6 mice (B) Kaplan-Meier survival analysis demonstrates no difference in overall survival for mice implanted with either GL261 (solid line) or GL261.luc cells (dashed line).

In conclusion, we believe that the viral transduction and expression of firefly luciferase in the GL261 mouse glioma cell line allows for an accurate, non-invasive imaging modality, which can provide an excellent surrogate for tumor volume in real-time. These cellular modifications had no significant effect on cell proliferation or invasion in cell culture, or invasion, immunologic cytokine profile, and animal survival, thus ensuring the efficacy and feasibility of BLI in this model.

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