

Monitoring Therapeutic Response in a Murine Model of Medulloblastoma Treated with a Small Molecule Inhibitor of Hedgehog Signaling

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Introduction

Medulloblastoma is embryonal in origin, malignant in nature, and the most prevalent brain tumor in children. Although the current standard of care (resection, radiation, and adjuvant chemotherapy) has improved 5-year progression free survival to nearly 70% [1], approximately 1/3 of patients are not cured and current therapies are associated with significant morbidity [2]. Therefore, alternative therapeutic approaches are strongly desired. Of particular interest are therapies that target molecular pathways shown to be deregulated in medulloblastoma, such as the hedgehog signaling pathway [1]. The work presented here has leveraged the non-invasive capability of small animal MRI to: 1) confirm medulloblastoma formation prior to initiation of treatment; and 2) monitor responses during therapeutic intervention with a small molecule inhibitor of hedgehog signaling (GDC-0449). These data show that MRI, when combined with pre-clinical pharmacokinetic data, may have utility for determining therapeutic dose and response in both preclinical and clinical settings.

Materials and Methods

All experiments were performed in accordance with institutional animal care and use guidelines. Male $Ptc1^{+/-}p53^{-/-}$ mice, a transgenic model of medulloblastoma [3], were used.

MRI was performed at 9.4T (Direct Drive; Varian, Inc., Palo Alto, CA). A multi-slice, T2 weighted fast spin echo sequence was used to visualize the cerebellum (TR/TEeff 4000/36 ms, FOV (2 cm)², matrix 128² zero-filled to 256², slice thickness 0.8 mm, NEX = 8; 4 cm transmit/receive volume coil; 8 minute acquisition). Animals were anesthetized using isoflurane. Body temperature was maintained at 36-37°C throughout imaging. Starting at approximately 5 weeks of age, mice were imaged biweekly until the presence of medulloblastoma was confirmed. Subsequently, animals were enrolled into treatment groups (vehicle, 12.5, 25, 50, 100, 150 mg/kg; b.i.d, p.o.; n = 3/group) and imaged weekly for two weeks at which point animals were euthanized. The presence of medulloblastoma and therapeutic response were verified histologically.

Results

The maximum penetrance estimate (51%) of the model was much lower than published literature values (95%, [3]). Typically, 4-6 imaging slices covered the extent of the cerebellum. The striated pattern of a healthy cerebellum in MR images (Figure 1) initially made determination of the presence of a medulloblastoma challenging. However, a brief pilot study comparing *in vivo* imaging with histology confirmed the accuracy of MRI-based diagnoses. GDC-0449 was well tolerated. Pre-treatment tumor volumes did not differ between groups. Vehicle treated animals had an approximate 2-fold increase in tumor volume over two weeks while GDC-0449 treatment resulted in a 42-96% decrease in tumor volumes (Figure 2). For doses ranging from 25 to 150 mg/kg, there were three animals with no quantifiable tumor volume by MRI after two weeks of treatment with GDC-0449 (Figure 3). Histology confirmed the absence of malignant masses and restoration of the folia architecture.

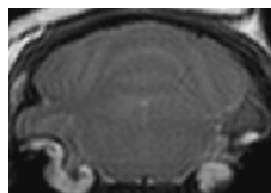


Figure 1: T2-weighted MR image showing the folia pattern of normal cerebellum.

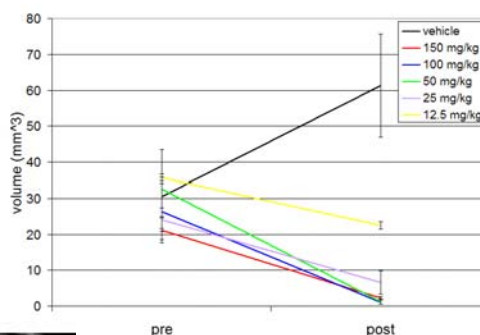


Figure 2: Volumetric measurements (mean ± SEM) made from MR images prior to and after two weeks of treatment with the GDC-0449.

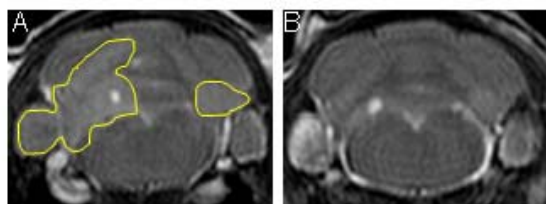


Figure 3: Example of complete responder (25 mg/kg) as measured by MRI. A) Yellow outlines demarcate presence of moderately sized medulloblastoma prior to treatment. B) Post-treatment image shows restoration of folia.

Discussion

The application of non-invasive MR imaging to this murine model of medulloblastoma had an impact in several ways. Medulloblastoma has rarely been monitored *in vivo* in a small animal model [4]. The reproducible image quality we were able to achieve made it easy to visualize the normal structure of the cerebellum and quantify tumor burden along with treatment response. Due to low penetrance, serial imaging became mandatory to confirm the presence of a tumor prior to treatment start in order to avoid performing a prevention study inadvertently. Without confirming the presence of a tumor in a given animal prior to initiating treatment, it is difficult to claim complete response in any small animal model of cancer with certainty. Future studies will include the use of gadolinium to assess blood brain barrier integrity pre- and post-treatment, varied duration of treatment regimens, and further histological confirmation of changes visualized by MRI.

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[1] Romer, et al. *Cancer Cell*. 2004, 6:229-40. [2] Gilbertson, et al. *Ann Rev Pathol Mech Dis*. 2008, 3:341-365. [3] Wetmore, et al. *Cancer Res*. 2001, 61:513-16. [4] Nelson, et al. *J of Neuro-Onc*. 2003, 62:259-67.