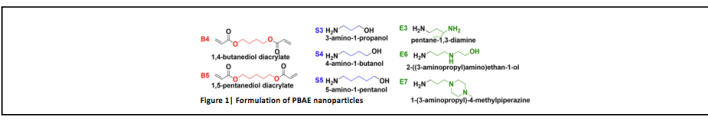


### Background and Study Objective

Together, medulloblastoma (MB) and atypical teratoid/rhabdoid tumors (AT/RT) represent two of the most prevalent pediatric brain malignancies in the youngest patients. Current treatment of these tumors typically involves radiation therapy, which carries significant risk of developmental sequelae, especially for patients under the age of three. In prior studies involving murine glioblastoma models, nanoparticles capable of carrying plasmids have shown promise as an alternative mode for delivering treatment. However, previous work with polymer based delivery of genes using polyethylenimine (PEI) demonstrated intolerable cytotoxicity in normal gray matter due to nonspecific polymer effects, thereby limiting its application. We thus set out to use less cytotoxic poly(beta-amino ester) (PBAE) nanoparticles for delivery of treatment in malignant pediatric brain tumors.



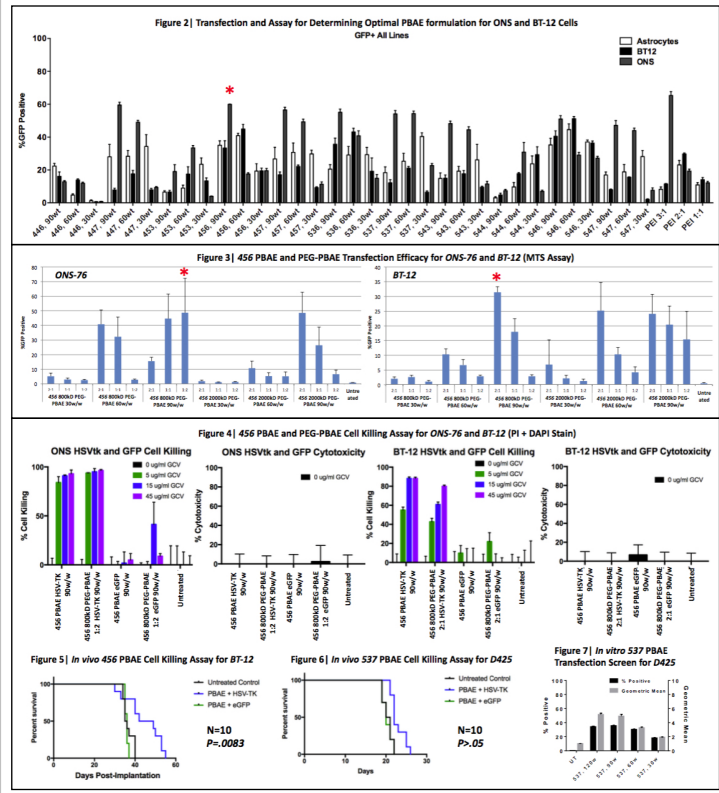
### Methods

Our interdisciplinary team investigated the efficacy of PBAE nanoparticles for delivering genes of interest such as HSV-TK to treat medulloblastoma and AT/RT. Experiments were first run *in vitro* using nanoparticles carrying eGFP or HSV-TK plasmids for both MB and AT/RT cell lines in order to test transfection efficacy/cytotoxicity and cell killing ability, respectively. Subsequent experiments in murine models implanted with MB and AT/RT were used to test efficacy and safety *in vivo*.

*In vitro*: ONS-76 and D425 (medulloblastoma) and BT-12 (AT/RT) cells were first each grown in cell culture and subsequently seeded in 96-well plates. Using MTS assay and flow cytometry, cells were assessed for optimal PBAE nanoparticle formulation through eGFP transfection. After selecting the optimal formulation (i.e. 456), cell killing efficacy of the nanoparticle was assayed using HSV-TK + ganciclovir (GCV) transfection with DAPI and PI staining.

*In vivo*: BT-12 cells ( $5 \times 10^5$ ) and group 3 medulloblastoma D425 cells ( $1.25 \times 10^5$ ) were implanted into the dorsal striatum of athymic nude mice and selected arms were treated with polymeric nanoparticles carrying the HSV-TK at 10, 20, and 30 days post-implantation for BT-12 implanted mice and 10 and 20 days for D425 implanted mice. GCV was administered daily following first treatment and extended for 10 days post-nanoparticle injection. Kaplan-Meier curves were generated and statistical tests were run to assess significance in overall survival between the following arms for both tumor groups: PBAE + HSV-TK (N=10), PBAE + eGFP (N=10), untreated controls (N=10).

### Results



### Results

*In vitro* studies using nanoparticle delivery of HSV-TK + ganciclovir in ONS-76 medulloblastoma models resulted in over 96% cell killing of cancer cells and over 90% cell killing in BT-12 AT/RT models.

*In vivo* AT/RT groups treated with intracranial convection enhanced delivery (CED) of PBAE HSV-TK nanoparticles with daily intraperitoneal ganciclovir injections had a greater mean overall survival (42 days) compared to untreated controls (35 days) ( $P=.0083$ ). Preliminary data from the *in vivo* D425 group 3 medulloblastoma group treated with CED of PBAE HSV-TK nanoparticles with daily ganciclovir injections demonstrated mean overall survival of 22 days compared to untreated controls at 20 days ( $P>0.5$ ).

### Limitations

Due to difficulty growing ONS-76 *in vivo*, a decision was made to test the more aggressive group 3 medulloblastoma cell line D425 instead. However, due to the non-adherent nature of D425 cells, the same screening methods we used for assaying transfection and cytotoxicity in ONS-76 and BT-12 provided suboptimal data. Also, the D425 implanted mice were only able to have two out of three planned particle injections (at days 10 and 14) due to the aggressive growth of the D425 cells *in vivo*.

### Conclusions and Future Steps

In regards to the data from the AT/RT cell line, this experiment does demonstrate proof of principle that non-viral gene therapy using polymeric nanoparticles can be used as a safe and effective treatment for certain pediatric brain malignancies.

However, further studies will be required to assess the efficacy of PBAE based treatment for medulloblastoma. A more complete and reliable transfection and cytotoxicity screen for D425 will be necessary to optimize PBAE polymer formulation for subsequent *in vivo* trials. Moreover, due to the aggressive growth of D425 cells *in vivo*, it would be beneficial to explore shortened durations between treatments.