

Berubicin: A Novel Topoisomerase II Inhibitor with Activity in Ependymoma

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Abstract

Introduction: Ependymomas are rare central nervous system tumors, occurring predominantly in children. Tumor size can make gross total resection exceptionally difficult, and post-operative radiation may be of benefit but CNS toxicity prevents its wide application in patients < 2 yrs. Development of new therapies for this tumor is limited by the absence of optimal *in vivo* and *in vitro* model systems. Drug therapy options are limited because few compounds can cross the blood-brain barrier, and almost all tumors of this type display significant resistance to chemotherapeutic agents.

Using an anthracycline scaffold and our own modular approach to designing DNA-binding agents, we have synthesized a chemical library of novel DNA binders. In screening this library against a panel of multidrug-resistant cell lines, we discovered novel structures, one of which was termed WP744 (AKA RTA 744 and Berubicin). Recent Phase I clinical studies in patients with glioblastoma demonstrated that Berubicin is active and crosses the blood brain barrier to reach therapeutic concentrations in the CNS. Clinical studies showed that the dose-limiting toxicity was myelosuppression, with a MTD of 7.5 mg/m²/day given 3 consecutive days every 21 days. Pharmacokinetic studies showed dose-independent clearance, with a mean half-life of 36-hours and large volume of distribution.

Methods: Assessment of cytotoxicity, growth inhibitory effects, and ability to induce apoptosis *in vitro* by berubicin in ependymoma and glioblastoma cell lines and comparison *in vitro* of berubicin with other topoisomerase II inhibitors.

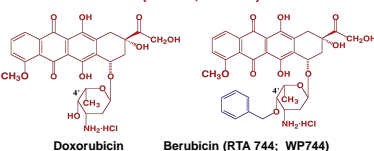
Results: These studies demonstrated the potent cytotoxic activity of berubicin against ependymoma which is superior compared to any other tested topoisomerase II inhibitor. The ependymoma cell lines tested were more sensitive to berubicin than glioblastoma cell lines. Specifically, Berubicin's IC₅₀ values ranged from low nM to low μM (picoMolar) levels (72 and 96 hr treatments). Berubicin also induced strong apoptotic response in glioblastoma and all ependymoma cell lines tested.

Conclusions: Berubicin, a clinically evaluated novel anthracycline demonstrates excellent *in vivo* activity in human brain tumors (primarily gliomas), possesses favorable drug-like properties, and subsequently shows superior activity *in vitro* against a variety of ependymoma cell lines. Together, these data provide a clear rationale supporting a clinical trial of Berubicin in patients with recurrent ependymal tumors.

Models Used

- Two Intracranial tumor cell line which can grow in mice (BT44, BT57)
- Five *in-vitro* cell lines established: Three human adherent lines (BT44, BT57, BT52), a human cancer stem-like cell line (CSC58), and mouse cell lines developed at St. Jude's shown below:
 - BRG247 – EphB2 tumor which histologically and genetically represents a subgroup of human ependymoma
 - BRG78 – Notch intracellular domain over expressing tumor generated from radial glial cells.
 - Wild Type – wild type embryonic (E14.5) radial glial cells, the putative cell of origin of ependymoma
 - Ink4a/ARF null - Ink4a/ARF null radial glial cells, the cell background of tumors BRG247 and BRG78

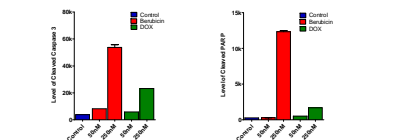
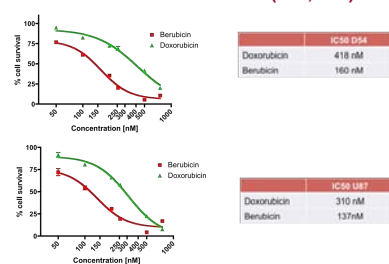
Structures of Berubicin (WP744, RTA744) and Doxorubicin



Please also see: R. Johnson et al. (2013). "Radial glia are susceptible to transformation into ependymoma by candidate human ependymoma tumor suppressor genes (TSO) and oncogenes." Abstract No. 517, Session: Primary Session V, Stem Cells Date Time: 10/22/2009, 10:25 AM - 10:35 AM

K. Wright et al. (Poster): "A comprehensive view of the structure and expression of the ependymoma genome." Abstract No.189, Session: Genetic/Genomics Poster session: 10/22/2009, 5:15 - 7:15 PM

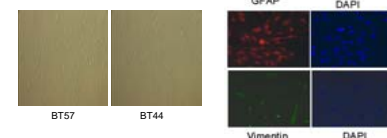
Berubicin is More Potent than Doxorubicin in Glioblastoma Cell Lines (D54, U87)



U-87 were seeded at 1x10⁶ cells per well in a 6-well plate and allow 24hr to attach. Berubicin and DOX were applied for 24hrs. 40ug of lysates were used on MSD plate

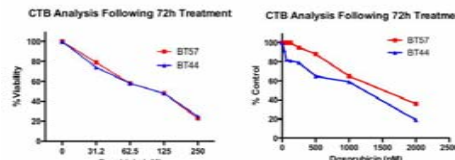
Establishment of *In-Vitro* Ependymoma Model

Ependymal cell lines, BT55 and BT44 grow in tissue culture as adherent cells. Immunofluorescence used as markers for ependymoma cells.

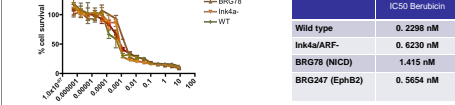
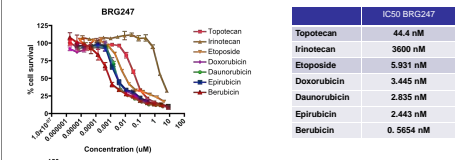


- An Intracranial and Subcutaneous model of ependymoma was established.
- The subcutaneous model showed an exponential growth of tumor mass.
- Both models showed Pseudorosette formation, classic features of ependymoma.
- Immunofluorescence staining showed GFAP and vimentin expression and can be used as markers of ependymoma

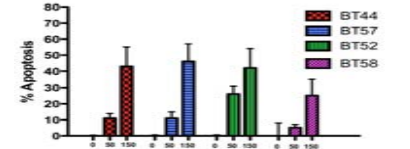
Berubicin Inhibits Adherent Ependymoma Cell Growth



Ependymoma cells at 5,000 cells/well on a 96-well plate in triplicate were treated with indicated concentrations of Berubicin for 72 hr. Cell growth was measured by Cell Titer Blue (CTB) and the line graph depicts the growth inhibition with Berubicin.

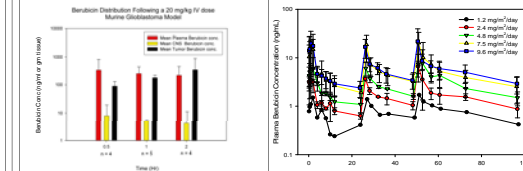


Berubicin Sensitizes Ependymoma Cells to Apoptosis



Treatment of Ependymoma cells with Berubicin induces apoptosis as detected by increased annexin V staining and as detected by flow cytometry.

Pharmacokinetics of Berubicin in Xenograft Mouse Models and Phase I Human Trials



In vivo berubicin concentration from 0.5 to 2 hrs in the specified biomarkers following a single 20 mg/kg IV dose. Error bars represent the standard deviation from the mean (Berubicin) within the sampled population.

- Preclinical studies have demonstrated berubicin penetrates the blood brain barrier, is delivered to brain tumor tissue, has predicted off-target effects, and efficacy in the treatment of CNS malignancies.
- Berubicin shows in Phase I clinical trials activity that ranged from stable disease to complete response, exceptional pharmacokinetic characteristics, and a manageable side effect profile.

Future Developments

Hematoxylin and Eosin staining tumor xenografts of ependymoma

Photomicrograph examples of ependymoma (panels "A" at 10 X and panel "B" at 40 X) implanted subcutaneous NuNu mice form tumors with characteristic histopathologic features of ependymoma. As can be seen (arrow), demonstrates a characteristic pseudorosette formation. This can also be seen in the higher powered panel B. Additionally, orthotopic xenograft models are being developed (data not shown) using human cancer stem-like cells (personal communication with Dr. Colman). These models should allow for *in vivo* drug testing.

Conclusions

- Berubicin (RTA 744; WP744), a novel anthracycline topoisomerase II inhibitor designed to cross blood-brain barrier by circumventing P-gp and MRP1 ABC transporters showed high preclinical activity in glioma *in vivo* models and promising activity in Phase I clinical studies
- Ependymoma cell lines and tumor samples express high levels of topoisomerase II thus we hypothesize ependymomas should be as sensitive as gliomas to Berubicin
- Surprisingly, ependymoma cell lines are more sensitive to Berubicin than glioma cell lines
- Berubicin is more potent, *in vitro*, than any other topoisomerase II inhibitors tested
- Berubicin more potently induces apoptosis in ependymoma, compared to glioma cell lines, and is a much more potent inducer of apoptosis than doxorubicin
- Preclinical PK studies and Phase I human trials in gliomas demonstrated that Berubicin penetrates the BBB, achieves cytotoxic concentrations in tumor tissues and is retained in tissue for a protracted period of time
- Collectively these data support the use of Berubicin in clinical trials for patients with ependymoma

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