DESIGN AND INITIATION OF AN ADAPTIVE, RANDOMIZED, CONTROLLED STUDY OF BERUBICIN, A TOPOISOMERASE II POISON THAT CROSSES THE BLOOD-BRAIN BARRIER FOR THE TREATMENT OF RECURRENT GLIOBLASTOMA MULTIFORME (GBM)

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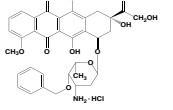
Abstract

Berubicin (also known as WP744) is a patented doxorubicin (Dox) analog with evidence that it crosses the BBB and has significant central nervous system (CNS) uptake. Induction of apoptosis and DNA damage by Berubicin was compared with Dox and showed much greater potency of Berubicin in all tested cancer cells. In addition, Berubicin has also shown consistently high cytotoxicity in GBM cell lines than significantly exceeded that of Dox. In models of intracranial orthotopic gliomas, Berubicin prolongs survival when compared to temozolomide, currently a standard of care in GBM. Evaluation of these models also show that Berubicin has greater infiltration of the tumor compared to normal tissue, providing additional support for improved efficacy.

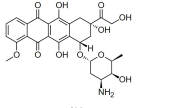
Based on this data, a Phase 1 dose escalation study was conducted in patients with recurrent primary brain tumors after a significant number of prior therapies. Berubicin was well tolerated, with myelosuppression (neutropenia and thrombocytopenia) as the dose-limiting toxicity. Of 25 patients evaluable for efficacy, there was 1 complete response (14+ years), 1 partial response durable for 12 weeks, and 9 patients with stable disease over 6 weeks for a clinical benefit rate of 44%.

CNS Pharmaceuticals, Inc. ("CNSP") licensed Berubicin and initiated a randomized, controlled clinical trial of Berubicin vs. Lomustine in adults with recurrent GBM after first line therapy. The primary endpoint of this study, being conducted in the United States and Europe, is overall survival (OS), with a projected 243 patients enrolled in a 2:1 randomization design (Berubicin:Lomustine). This study has pharmacokinetic (PK) evaluations of all patients enrolled, with at least 15 patients undergoing complete PK assessments throughout the initial dosing period (3 days of IV administration of Berubicin over 2 hours, repeated every 3 weeks). Patients will be stratified on the basis of MGMT methylation, there will be documentation of IDH mutational status, and no prior administration of bevacizumab will be allowed. An interim analysis will evaluate the comparative effectiveness of these treatments, an adaptive design intended to demonstrate that Berubicin's efficacy is at least equal to that of Lomustine (futility analysis). The overall survival endpoint and sample size have been calculated to be able to show a statistical difference between the two therapies as second line treatment for GBM.

Additional studies in malignant diseases of the CNS (e.g., pediatric brain tumors, primary CNS lymphoma, metastatic tumors) are also being developed based on preclinical data as well as the potential for activity of an anthracycline that penetrates the BBB in these indications. For the present study posted on clinicaltrials.gov as NCT04762069, additional details and contact information is available.



Berubicin



Doxorubicin

Berubicin is more potent than Doxorubicin in Solid Tumors and Lymphoma Cell Lines

Cell Line	Tumor Type	Berubicin IC$_{50}$(nM)	Doxorubicin IC$_{50}$(nM)	Ratio
MCF-7	Breast	0.72	4.02	5.6
NCI-H522	Lung	3.40	8.32	2.4
A549	Lung	1.07	4.68	4.4
SW480	Colon	2.99	8.26	2.8
HT-29	Colon	3.66	23.40	6.4
AsPC-1	Pancreas	5.62	80.50	14.3
BxPC-3	Pancreas	4.05	15.70	3.9
Capan-1	Pancreas	5.30	30.75	5.8
OVCAR-3	Ovarian	5.31	11.53	2.2

Cell line	Tumor Type	Berubicin IC$_{50}$(nM)	Doxorubicin IC$_{50}$(nM)	Ratio
Toledo	B cell lymphoma	3.1	24.5	7.9
HD4	Hodgkin's B cell lymphoma	4.0	43.0	10.7
HD2	Hodgkin's T cell lymphoma	5.2	53.0	10.1
HH	Cutaneous T cell lymphoma	11.6	57.4	4.9
MJ	Cutaneous T cell lymphoma	10.0	40.0	4.0
Daudi	Burkitt lymphoma	3.4	12.2	3.6

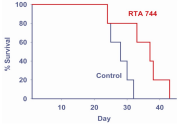
Comparison of IC50values for Berubicin and doxorubicin (Ratio = IC50Dox/ IC50Berubicin)

Berubicin is more potent than Doxorubicin in Primary CNS Tumor Cell Lines

Cell Line	Tumor Type	Berubicin (nM)	Doxorubicin (nM)	Ratio
D556	Human Medulloblastoma	5.1	36.0	7.0
DAOY-WT	Human Medulloblastoma	13.0	47.0	3.6
GL261	Murine Glioma	11.5	46.2	4.0
U-251	Glioblastoma	4.7	62.3	13.3
U-87	Glioblastoma	50.3	163.8	3.3
BT-58	Ependymoma	36.5	124.0	3.4

Comparison of IC50values for Berubicin and doxorubicin Ratio = IC50Dox/ IC50Berubicin

Berubicin Increases Survival in U-87 Mouse Orthotopic Glioma Model



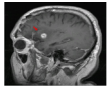
U-87 MG (human glioblastoma) cells implanted into the right hemisphere of the brain in nu/nu mice. Day 1 and Day 10. 10 mg/kg Berubicin or vehicle were delivered by i.p. injection.

Phase 1 Clinical Trial

Thirty-five patients with recurrent or refractory GBM or other primary brain cancers received IV Berubicin over 2 hours for 3 consecutive days (one cycle) every 21 days. Doses were escalated using an accelerated titration design and ranged from 1.2 to 9.6 mg/m^2/day.

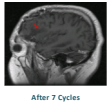
The most common dose limiting toxicity (DLT) was myelosuppression, specifically neutropenia. Minimal nonhematological toxicities were observed, no neurotoxicity or cardiotoxicity was noted. The maximum tolerated dose (MTD) of Z. Muzyczenko1 was determined to be 7.5 mg/m^2/day.

Of 25 patients evaluable for efficacy, one patient demonstrated a Complete Response (CR) and is in remission >13 years; 2 patients had partial/minor responses; 9 patients had stable disease; with an overall 48% clinical benefit rate.



Baseline

- MRI scan of GBM (confirmed) patient at baseline, then after 7 cycles of Berubicin
- The patient had a complete response (CR) to treatment with Berubicin
- The patient remains in remission 13 years later despite no additional therapy



After 7 Cycles

Protocol Design and Objectives

Protocol Title	Multicenter, Open-Label Study with a Randomized Control Arm of the Efficacy, Safety, and Pharmacokinetics of Intravenously Infused Berubicin in Adult Patients with Recurrent Glioblastoma Multiforme (WHO Grade IV) After Failure of Standard First Line Therapy
Indication	Recurrent Glioblastoma Multiforme (GBM) after failure of first line therapy
Primary Objective	To assess the effect of Berubicin compared with Lomustine on overall survival (OS) in adult patients with GBM (WHO Grade IV) after standard initial therapy
Primary Endpoint	Overall Survival
Number of Patients	210 2:1 randomization (Berubicin:Lomustine)
Interim Analysis	Comparative interim analysis: ~ 30 % of all patients at 6 months for futility
Berubicin Administration	7.5 mg/m^2 as a 2-hr infusion Days 1-3 every 21 days
MRI scans	Every 6 weeks evaluated by a Central Reader
Eligibility Criteria	Only one prior therapy for disease with documented recurrence; no prior Lomustine; stratified by MGMT status, requirement for IDH WT identification

Operational Highlights

- Acquiring Regulatory Approvals: Fast Track and Orphan Drug Status (US)
- Incorporating 2021 WHO classification (IDH Mutational Status) to define eligibility
- Stratifying based on MGMT methylation status
- Requiring Central Read of Scans
  - Advanced imaging technology
  - Validation of eligibility and response evaluation (secondary endpoints)
- Allowing re-resections prior to enrollment
- Using Patient-Reported Outcomes (PRO)
- Generating Population PK throughout the study
- Sourcing and providing Lomustine by sponsor
- Performing a Global Study:
  - US: Arizona, Arkansas, California, Colorado, Florida, Georgia, Illinois, Kentucky, Louisiana, Massachusetts, Minnesota, Missouri, Nebraska, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Texas, Utah, Washington, Wisconsin
  - EU: Switzerland, Spain, France, Italy