Doxorubicin Response to Temozolomide-resistant Brain Cancer Glioblastoma Cells

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Background & Significance

- Glioblastoma Multiforme (GBM) is the most aggressive primary neoplasm of the CNS, accounting for approximately 60% of all primary brain tumors with 12,500 newly diagnosed cases in the US annually.
- Standard of care includes surgery, radiotherapy, and temozolomide (TMZ) chemotherapy.
- Drug resistance against TMZ occurs because of the unmethylation of MGMT, a DNA repair enzyme.
- Methylation of the MGMT promoter region of the DNA repair enzyme by TMZ inactivates the enzyme resulting in survival benefit of GBM patients (Fig. 1).

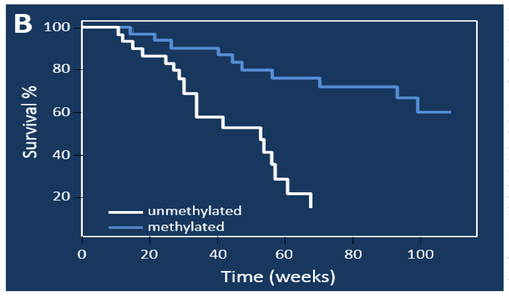


Figure 1: Kaplan-Meier diagram depicting overall survival of patients according to the MGMT promoter methylation status. *Nagasawa et al, 2012*

Rationale

- Doxorubicin is a highly effective therapeutic agent for the treatment of many malignant tumors.
- Several different formulations of doxorubicin have been developed, but lack of penetration through the blood brain barrier (BBB) limits their use in the treatment of brain tumors.
  - Dr. Prakash and his colleagues have shown potent anti-GBM activity of a novel drug – aldoxorubicin, which is an albumin binding doxorubicin (Neoplasia 2014; 16:874)
  - This provided strong rationale for evaluating this drug as a treatment for patients with GBM.

Hypothesis

TMZ and doxorubicin induce DNA damage by different mechanisms. Thus, it is our hypothesis that TMZ-resistant GBM cells will remain sensitive to the cytotoxic effects of doxorubicin.

Figure 2: Aldoxorubicin Structure

Aldoxorubicin (INNO-206) is a prodrug of the anti-cancer agent doxorubicin which is derivatized at its C-13 keto-position with a thiol-binding spacer molecule (6-maleimidocaproic acid hydrazide).

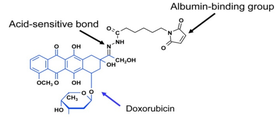
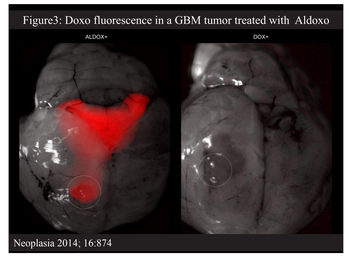


Figure3: Doxo fluorescence in a GBM tumor treated with Aldoxo



Neoplasia 2014; 16:874

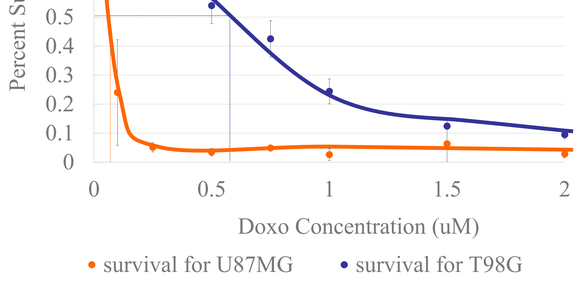
Table 1: Genetic characteristics of different cell lines

Xenograft or cell line	U87*	T98*	GBM12-Arg**	GBM12TMZ**
Temozolomide (TMZ)	sensitive	resistant	sensitive	resistant
IC_{50} for TMZ (uM)†	23	441.6	N/A	N/A
MGMT Promoter	methylated	unmethylated	N/A	N/A
Mouse survival (days)^	N/A	N/A	68	43

*Neoplasia 2011;13:537 **Mol Cancer Ther 2009; 8:407 †International J. Onc. 2010;36:1367
^TMZ treatment evaluation in intracranial GBM12 and GBM12TMZ xenograft mouse model

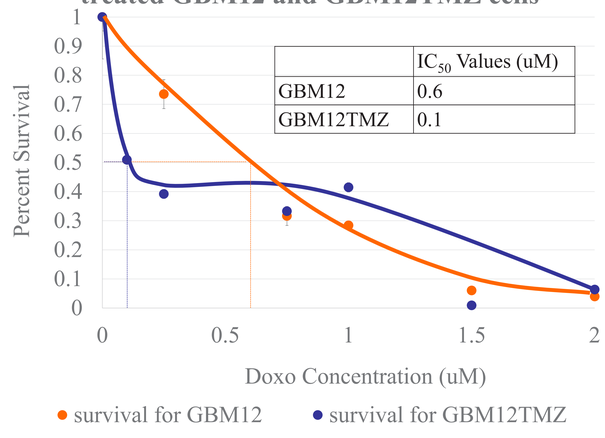
Results

Figure 4: Percent Survival in Doxo-treated U87MG and T98G cells



	IC_{50} Values (uM)
U87MG	0.07
T98G	0.57

Figure 5: Percent Survival in Doxo-treated GBM12 and GBM12TMZ cells



	IC_{50} Values (uM)
GBM12	0.6
GBM12TMZ	0.1

Figure 6: Doxo retention in U87 and T98 cells

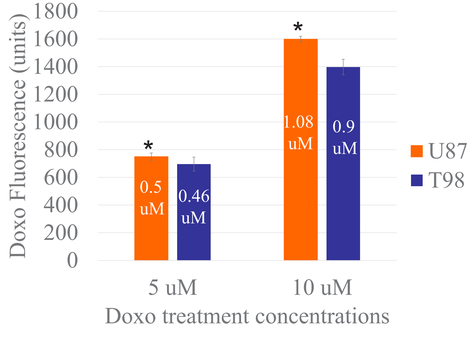
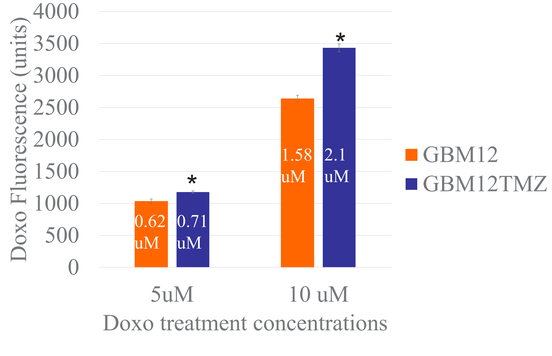


Figure 7: Doxo retention in GBM12 and GBM12TMZ cells



- We compared doxorubicin sensitivity in human GBM U87 (TMZ-sensitive) and T98G (TMZ-resistant) cells and in GBM12 (TMZ-sensitive) and GBM12TMZ (TMZ-resistant) xenograft lines.
- Although T98 cells were less sensitive to the cytotoxic effects of doxorubicin compared with U87, their survival rapidly dropped with increasing drug concentrations.
- GBM12TMZ xenograft line was significantly more sensitive to doxorubicin compared with GBM12 cells.
- Poor drug retention was not the factor in higher doxorubicin resistance observed in T98 and GBM12 cells because the drug retention was comparable to that in U87 and GBM12TMZ cells.

Conclusions & Translational Relevance

Our preliminary study suggests that doxorubicin can be an effective cytotoxic agent in TMZ resistant GBM cells and provides a strong rationale for evaluating the efficacy of aldoxorubicin, the albumin-binding doxorubicin (Fig.2) in preclinical TMZ-resistant GBM tumor cells and in clinical studies in patients with recurrent GBM.

Acknowledgements

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