



# Pharmacological and Pre-Clinical Testing of 5-NiDR as a New Therapeutic Agent Against Brain Cancer

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## ABSTRACT

Approximately 4,000 children in the United States are diagnosed annually with a brain tumor. Brain cancers are the deadliest of all pediatric cancers as they have survival rates of less than 20%. Although surgery and radiation therapy are widely used to treat adult patients, chemotherapy is the primary therapeutic option for children. One important chemotherapeutic agent is temozolomide, an alkylating agent that causes cell death by damaging DNA. In this project, we tested the ability of a specific non-natural nucleoside developed in our lab, designated 5-NiDR, to increase the efficacy of temozolomide against brain cancer. Cell-based studies demonstrate that the combination of 5-NiDR and temozolomide kills more cells compared to treatment with either temozolomide or 5-NiDR used alone. Microscopy techniques demonstrate that the combination of 5-NiDR and temozolomide causes cell death via apoptosis rather than necrosis. Animal studies using xenograft (nude) mice were performed to evaluate the *in vivo* efficacy and safety of this drug combination against brain cancer. Preliminary results are provided which indicate that treatment with 5-NiDR does not inhibit the rate of tumor growth. In contrast, treatment with temozolomide reduces the rate of tumor growth but does not lead to the complete elimination of the tumor. Striking results are obtained using 5-NiDR and temozolomide together as this drug combination causes a significant reduction in tumor size. Finally, mice treated with the combination of 5-NiDR and temozolomide do not show overt signs of side effects such as weight loss, dehydration, or fatigue. Collectively, these studies provide pharmacological evidence for combining 5-NiDR and temozolomide as a new treatment strategy to effectively treat brain cancers.

## INTRODUCTION

Glioblastoma is the most common and aggressive form of brain cancer. While there are several options to treat this type of brain cancer, each has significant disadvantages. For example, surgery can reduce tumor burden but it can not completely remove all cancer cells. Focal radiation therapy can also eradicate cancer cells. However, this option can cause side effects by damaging normal brain tissue. Chemotherapy is often the final option and is used to treat adult and pediatric patients.

Of all chemotherapeutic agents, temozolomide is the most effective agent used against brain cancers such as glioblastoma. Temozolomide is an orally administered alkylating agent that can effectively pass the blood-brain barrier to damage brain cancer cells. This agent preferentially reacts with guanine residues in DNA to create two distinct lesions, O<sup>6</sup>-methylguanine and abasic sites. Both lesions can inhibit DNA synthesis which subsequently induces cell death. Unfortunately, these lesions can also be misreplicated in a process called translesion DNA synthesis (Figure 1). This process can limit the efficacy of temozolomide by causing drug resistance and increasing the frequency of mutagenesis.

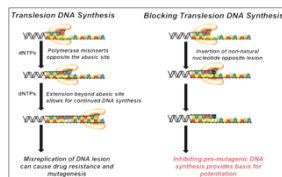


Figure 1. (left panel) DNA polymerases can misreplicate lesions created by temozolomide. This activity causes drug resistance and increases mutagenesis. (right panel) Strategy to use non-natural nucleosides to inhibit the misreplication of lesions produced by temozolomide.

To combat these problems, we developed a novel non-natural nucleoside designated 5-NiTP (Figure 2) which is efficiently and selectively inserted opposite abasic sites during translesion DNA synthesis. After insertion opposite the DNA lesion, this non-natural nucleoside inhibits the ability of DNA polymerases to extend beyond the abasic site. This inhibition is predicted to increase the potency of temozolomide, thus making the chemotherapeutic more effective against brain cancers.

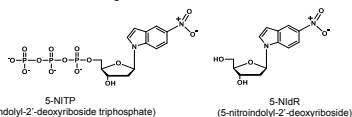


Figure 2. Structures of the non-natural nucleoside, 5-NiTP, and corresponding nucleoside, 5-NiDR designed to inhibit the cellular misreplication of DNA lesions produced by temozolomide.

## GOALS

1. To define the anti-cancer activity of 5-NiDR as a monotherapeutic agent against brain cancer.
2. To determine if 5-NiDR increases the potency of temozolomide by lowering its LD<sub>50</sub> value.
3. To perform animal studies that evaluate efficacy and safety of this drug combination (5-NiDR + temozolomide).

## In vitro studies

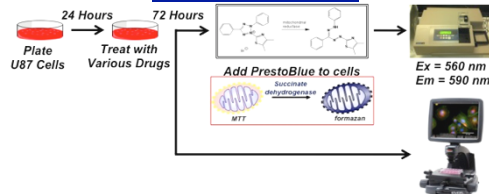


Figure 3. The viability of brain cancer cells is quantified using PrestoBlue assay and visualized by fluorescence microscopy.

## I. RESULTS

Figure 4. Representative dose-response curves for the cytotoxic effects of (A) 5-NiDR, (B) temozolomide (TEM), and (C) TEM with 5-NiDR. U87 brain cancer cells were treated with variable concentrations of agent for three days and then analyzed for cell death. LD<sub>50</sub> values were measured using the following equation:

$$\% \text{ viable cells} = 100 / (1 + (LD_{50}/[\text{agent}])^n)$$

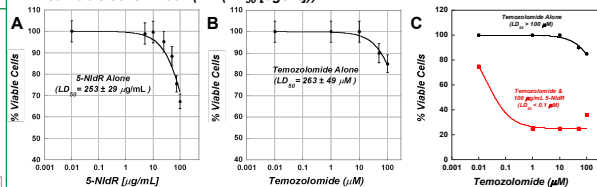
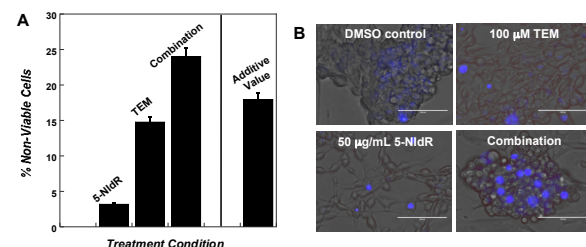


Figure 5. (A) Data showing % non-viable cells after treatment with 5-NiDR, TEM, or TEM combined with 5-NiDR. The combination of TEM and 5-NiDR is synergistic as it kills more cells than the additive effects of 5-NiDR and TEM alone. (B) Microscopic analysis validates that treatment with TEM and 5-NiDR produces a different cellular morphology than 5-NiDR or TEM alone.



## CONCLUSIONS

1. 5-NiDR alone displays weak potency against brain cancer cells.
2. Combining sub-lethal doses of 5-NiDR and temozolomide causes synergistic anti-cancer effects against brain cancer cells.
3. The combination of 5-NiDR and temozolomide causes significant reduction in tumor size in xenograft mice.
4. No overt side effects are observed in mice treated with 5-NiDR alone or in combination with TEM.

## FUTURE DIRECTIONS

1. To determine the cellular mechanism(s) for how 5-NiDR increases the effectiveness of temozolomide.
2. To further optimize the dosing regimen for combining 5-NiDR with temozolomide.
3. To perform histological and biochemical analysis to validate the anti-cancer activity of combining 5-NiDR with temozolomide.

## In vivo studies



Figure 6. Xenograft (nude) mice were injected with ~5 million cancer cells on the right hind flank. After the tumor reached ~150 mm<sup>2</sup>, drug injections were performed and tumor growth was monitored daily. Weight measurements were taken every two days. Mice were euthanized when the tumor volume is larger than 1,500 mm<sup>3</sup>.

## II. RESULTS

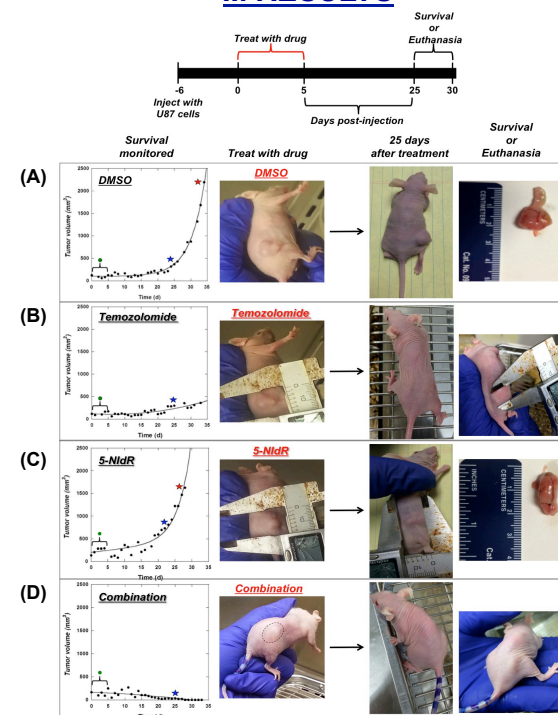


Figure 7. Treatment with temozolomide slows down tumor growth more significantly than treatment with 5-NiDR alone. However, combining 5-NiDR with temozolomide causes complete tumor regression. This result provides a new treatment strategy to combat brain cancers.

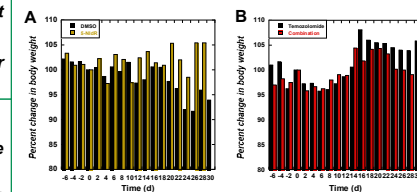


Figure 8. 5-NiDR does not produce overt toxic side effects. This is evident as mice treated with 5-NiDR alone or with TEM do not lose weight over the course of their treatment.

## ACKNOWLEDGMENTS

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