

## Abstract

Toca 511 (vocimagene amiretrorepvec), an amphotropic retroviral replicating vector (RRV), can successfully and safely deliver a functional, optimized yeast cytosine deaminase (CD) gene to tumors. Within infected cells, CD converts 5-fluorocytosine (5-FC) to the anti-cancer drug 5-fluorouracil (5-FU). The combination of Toca 511 with oral extended release 5-FC (Toca FC), is currently being evaluated in a randomized phase III clinical trial for recurrent high grade glioma (glioblastoma (GBM) and anaplastic astrocytoma) (NCT02414165, Toca 5). Temozolomide (TMZ), in combination with radiation therapy, after surgery is the standard of care used for first-line chemotherapy treatment of patients with GBM, the most common and aggressive form of primary brain cancer. Previously, we have shown that: (1) Toca 511/5-FC treatment provides durable response in a syngeneic murine glioma model and supports anti-tumor immune memory; (2) The combination of TMZ and Toca 511/5-FC had synergistic efficacy in a TMZ-sensitive human glioma nude mouse model; (3) TMZ did not inhibit the efficacy of Toca 511/5-FC in a TMZ resistant murine glioma syngeneic model; (4) Toca 511/5-FC caused significant radiosensitization in a radioresistant murine glioma model.

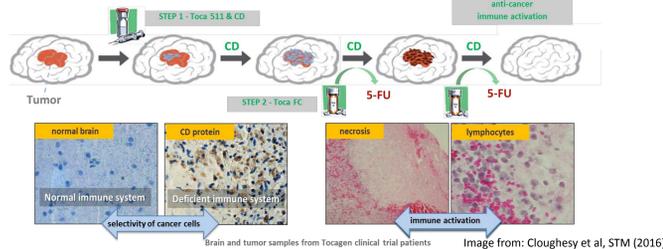
**Results:** To assess the interaction of TMZ with escalating doses of Toca 511 (as defined by percent of tumor transduction by RRV), an orthotopic TMZ-resistant murine glioma model, Tu-2449, was utilized. These results show that moderate levels of tumor transduction of Toca 511 (30% - 50%) with 5-FC treatment longer survival in the presence of TMZ compared with lower transduction rates (10%).

**Conclusion:** These results demonstrate that (1) survival associates with the transduction levels of Toca 511 when combined with TMZ in the Tu-2449 orthotopic glioma model and (2) that this combination may support anti-tumor immune memory. These studies along with prior work support evaluation of the combination of Toca 511/5-FC with TMZ in patients with newly diagnosed GBM (NRG-BN006).

## Introduction

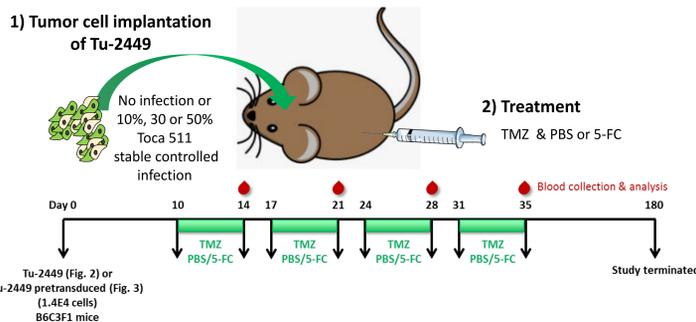
Toca 511 is a retroviral replicating vector (RRV) expressing a cytosine deaminase (CD) gene.

- Selectively infects tumor cells
- Buds off from but does not lyse tumor cells directly
- CD converts 5-FC (5-fluorocytosine) into anti-cancer drug 5-FU (5-fluorouracil)
- Immune system is activated selectively against the tumor



## Methods

### Tu-2449 Intracranial Tumor Model



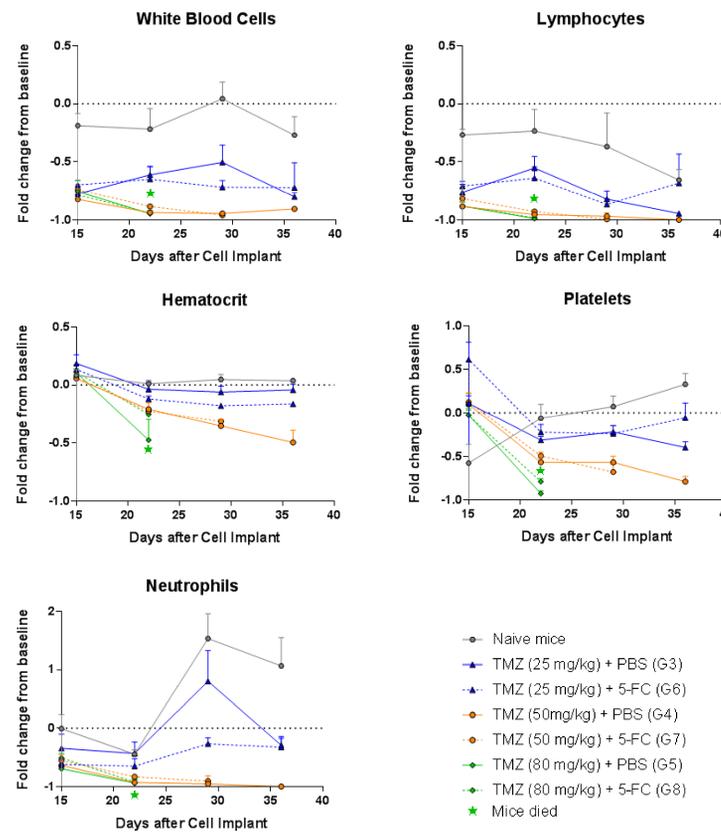
**Figure 1:** Schematic of TMZ resistant intracranial model. Toca 511 and Toca GFP infected Tu-2449 were admixed prior to intracranial implantation to achieve indicated stable infection rates. Mice were treated IP with 25 mg/kg TMZ with or without 5-FC for 4 cycles starting on day ten post implantation (5 days on and 2 days off). Blood analysis was performed following the last day of each cycle.

#### Study Design

- Tu-2449 cells were first 100% infected in vitro with Toca 511 or a sister vector that expresses GFP (green fluorescent protein) instead of CD.
- In order to control Toca 511 spread, these cells were then admixed at various percentages as Toca 511 does not readily infect cells already infected with the GFP virus.
- Naive B6C3F1 mice were implanted intracranially with the admixed Tu-2449 with either 10%, 30% or 50% Toca 511-infected cell mix.
- Cycles of 500 mg/kg 5-FC and 25 mg/kg TMZ were started on day 10 after intracranial tumor implantation (Figure 1).

## Results

### High concentrations of TMZ contribute to depletion of WBC, lymphocytes and neutrophils in mice



	Day	HCT	Plt	Neu	WBC	Lym
Vehicle	PBS	14				
	21					
	28					
	35					
	35					
25 mg/kg	PBS	14				
	21					
	28					
	35					
	35					
50 mg/kg	PBS	14				
	21					
	28					
	35					
	35					
80 mg/kg	PBS	14				
	21					
	28					
	35					
	35					

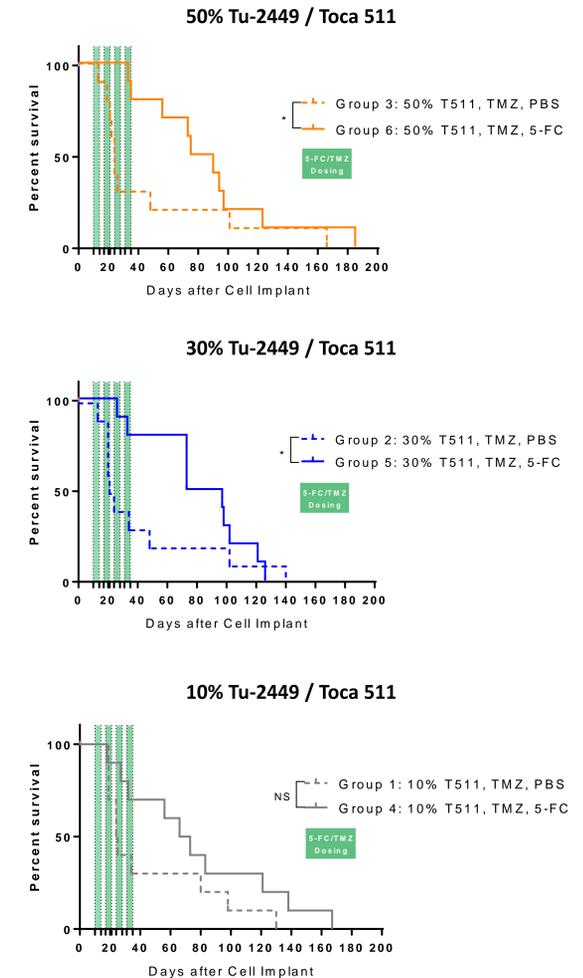
**Figure 2:** (top) Blood collections from Tu-2449 non-transduced mice were conducted weekly at the end of each cycle of treatment and complete blood count (CBC) panel was performed for white blood cell count (WBC), lymphocytes (Lym), hematocrit (HCT), platelets (Plt) and neutrophils (Neu). Baseline values for each animal were assessed prior to Tu-2449 cell implantation. (bottom) Statistical analysis of blood was performed; T-test compared to baseline of treated animals (t-test, 2-tailed, unequal variance). In chart above, significance of P values is indicated by color.

#### Blood analyses showed:

- As TMZ concentration increased, mice became more immunosuppressed and perished before the third week of treatment.
- TMZ contributes to hematological toxicity; whereas, 5-FC does not create additional toxicity.
- Addition of low dose TMZ (25 mg/kg) had lower effect on blood cell populations than higher doses.
- Blood collection from animals show low levels of WBC, lymphocytes and neutrophils in line with a leukopenia phenotype.
- Platelets and hematocrit were largely unaffected by TMZ.

## Results

### The addition of Toca 511 + 5-FC to TMZ improves survival



Group	Group Name	Median Survival (days)	Significance	P value
1 vs 4	10% T511, TMZ, PBS vs 10% T511, TMZ, 5-FC	24.5 vs 69.5	NS	0.126
2 vs 5	30% T511, TMZ, PBS vs 30% T511, TMZ, 5-FC	22.5 vs 85	*	0.025
3 vs 6	50% T511, TMZ, PBS vs 50% T511, TMZ, 5-FC	24 vs 82.5	*	0.015

**Figure 3:** Survival graphs and statistical significance details of 10, 30 and 50% Toca 511 and 25 mg/kg TMZ +/- 5-FC. Addition of 5-FC to TMZ at 30% & 50% of Toca 511 infection provided a statistically significant survival benefit with a p value of 0.025 and 0.015, respectively.

## Conclusion

- 5-FC combined with lympho-depleting TMZ provides survival benefit at 30% and 50% Toca 511 infection.
- TMZ depletes various blood populations, including white blood cells, lymphocytes and neutrophils.
- Optimization of the immune-associated mechanism of Toca 511 & Toca FC and the anti-tumor activity of TMZ in combination will require considerations of timing on the dosing schedules of the agents.
- Data from this study may inform and help design future clinical development of Toca 511 and Toca FC with TMZ standard of care in GBM.

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