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# INNOVATIVE STRATEGIES IN BRAIN CANCER DRUG DISCOVERY AND TARGETED DELIVERY: ADVANCES AND CHALLENGES IN PRECISION MEDICINE

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**Abstract**

Brain cancer is still among the most difficult malignancies to treat because of the complexity of blood-brain barrier (BBB), heterogeneity of tumors and low effectiveness of currently implemented drugs. In India, glioblastoma multiforme (GBM) is a disease that is increasingly becoming a major cause of CNS tumor and there is an urgent need to formulate the precision medicine-based drug discovery and targeted delivery models. This paper analyses in vitro and preclinical metadatas of a newly developed polymeric nanoparticle-based targeted drug delivery system of temozolomide (TMZ), the current standard-of-care drug of GBM. PLGA was conjugated with transferrin ligands in order to develop nanoparticles that improve BBB penetration and uptake in tumor cells. There was an evaluation of drug loading, release kinetics, cytotoxicity (U87MG cell line) and BBB permeation in an in vitro Transwell model and preclinical pharmacokinetic and tumor regression studies in Wistar rats with induced orthotactic gliomas. The findings showed an improved drug loading, longer release, 2.5 times increased BBB permeability and a much better tumor suppressive activity in comparison with free TMZ. This experimental study is translational in nature and it focuses on India with relevance to the pharmacological use of the ligand-based nanocarriers in the management of brain cancer.

**Keywords:** targeted drug delivery, brain cancer, nanoparticles, precision medicine, blood- brain barrier, glioblastoma.

## Introduction

One of the most aggressive and deadly types of cancers is brain tumors, especially glioblastoma multiforme (GBM), and its median survival is less than 15 months even with surgery, radiotherapy, and chemotherapy (Thakkar et al., 2014). The big therapeutic problem is that most drugs are unable to penetrate the blood and brain barrier (BBB) which is a selective barrier limiting the penetration of a drug into tissues in the brain (Pardridge, 2012).

Recent epidemiological published in India indicate that there is a statistically significant increase in GBM cases in the country, especially in urban tertiary care facilities (Srinivasan et al., 2020). Traditional systemic chemotherapy with temozolomide (TMZ) has some limitations of fast systemic elimination, non-specific toxicity, and poor delivery to tumor locations.

Nanotechnology-based targeted drug delivery system to boost BBB penetration and tumor selectivity and minimize systemic side effects is the current target of emerging precision medicine techniques (Bregy et al., 2013). Polymeric nanoparticles, which are conjugated with targeting ligands (e.g., transferrin, folate) are also promising in preclinical studies.

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**Background of the Study**

The discovery of drug targets in brain cancer has taken a new direction across the world through a combination of molecular targeting, drug repurposing, and nanotechnology to eliminate therapeutic resistance and enhance efficacy (Omuro & DeAngelis, 2013). In India, the majority of the patients are handled using standard procedures and local innovation in targeted delivery is not prevalent.

Targeting of transferrin receptors is most encouraging in brain tumors since the transferrin receptors on BBB endothelial cells and glioma cells are over expressed. Nanoparticles of poly lactic-co-glycolic acid (PLGA) have biocompatibility, controlled release, and accepted regulation (Danhier et al., 2012).

**Justification**

Although there are good prospects in the world, shortage of India specific preclinical studies to prove the functionality of targeted nanocarrier systems to brain cancer exists. This study is justified by:

- The increased GBM prevalence in clinical settings in India.
- The dire necessity of efficient medications delivery methods that can be used with the Indian healthcare infrastructure.
- The possibility of locally produced and modified to clinical application of the cost-effective nanocarriers based on PLGA.

**Objectives**

- To formulate and describe the PLGA-transferrin nanoparticle preparations of temozolomide.
- To evaluate in vitro drug release, cytotoxicity and BBB permeability as against free TMZ.
- To determine preclinical pharmacokinetics and therapeutic efficacy of an orthotopic glioma rat model.
- To give precision drug delivery in brain cancer experimental evidence with India focus.

**Literature Review**

Various preclinical trials across the world have demonstrated that ligand-targeted nanoparticles can have a major impact in improving brain drug delivery (Bregy et al., 2013; Huang et al., 2016). The application of PLA nanoparticles is in large quantities due to its biodegradability and controlled release capability (Danhier et al., 2012).

Transferrin-functionally loaded nanoparticles were found to be 23 times more permeable to the BBB in both in vitro and in vivo models (Kreuter, 2014). Only very few scholarly works have explored the delivery of nanoparticles as TMZ in India, mostly confined to physicochemical profiling (Bharadwaj et al., 2018). An obvious lack of integrated in vitro and preclinical evaluation is present.

**6. Materials and Methodology**

**6.1 Nanoparticle Preparation**

Emulsion solvent evaporation was used to prepare PLA nanoparticles which were loaded with TMZ. Transferrin was EDC/NHS conjugated. Blank and non-targeted PLGA nanoparticles were made as controls.

The physicochemical characterization of the product was conducted using

**6.2 physicochemical characterization**

- Particle size & PDI: DLS
- Zeta potential: Electrophoretic Light scattering.
- Drug loading/ entrapping: Post dissolution UV Vis spectrophotometry.

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**6.3 In Vitro Drug Release**

The suspension of nanoparticles was carried out in pH 7.4 (37 o C). The samples were sampled until 120 h and the content of TMZ was analysed.

**6.4 Cytotoxicity (MTT Assay)**

U87MG glioblastoma cells were exposed to free TMZ, PLGA-TMZ, and PLGA-Tf-TMZ (transferrin) of different concentrations after 48 h.

**6.5 BBB Permeation Assay**

A Transwell in vitro BBB system was developed on human brain microvascular endothelial cells (HBMeCs) co-culture with astrocytes. Formulations permeability was determined by evaluating TMZ concentration in basolateral chamber after 6 h.

**6.6 Preclinical Study**

Free TMZ or nanoparticle formulations were given intravenously to Wistar rats that had orthotopic gliomas induced. To perform pharmacokinetic analysis (LC–MS), tissues and blood were taken (brain and blood). In 21 days, tumor regression was observed using MRI.

**6.7 Statistical Analysis**

Each of the experiments was repeated 3 times. One-way ANOVA was used to analyze data ($p < 0.05$ was taken as significant).

**7. Results and Discussion**

**7.1 Physicochemical Characterization**

The PLGA-transferrin nanoparticles demonstrated favorable physicochemical properties suitable for brain-targeted drug delivery (Table 1).

**Table 1. Physicochemical properties of nanoparticle formulations**

| Parameter | PLGA-TMZ (Non-targeted) | PLGA-Tf-TMZ (Targeted) |
|---|---|---|
| Particle size (nm) | 165 ± 12 | 178 ± 10 |
| PDI | 0.19 ± 0.02 | 0.21 ± 0.03 |
| Zeta potential (mV) | −18.5 ± 1.2 | −22.3 ± 1.4 |
| Drug loading (%) | 9.2 ± 0.5 | 10.1 ± 0.6 |
| Entrapment efficiency (%) | 74.5 ± 3.1 | 81.2 ± 2.8 |

**Key findings:**

The targeted nanoparticles were slightly bigger in size because of conjugation with transferrin but could still be transported by the BBB because they were less than 200 nm. It was found that the transferrin-functionalized nanoparticles could be loaded with higher efficiency and entrapment and this is in line with the encapsulation stability (Danhier et al., 2012). In Vitro drug release profile was studied by putting the marble into an acetone solution and incubating it at various temperatures across different durations (7.2 In Vitro drug release profile was investigated by placing the marble into an Acetone solution and incubating it in different temperatures over different periods (7.2).

Figure 1 illustrates the drug release profile of TMZ of PLGA and PLGA-Tf nanoparticles against that of free drug.

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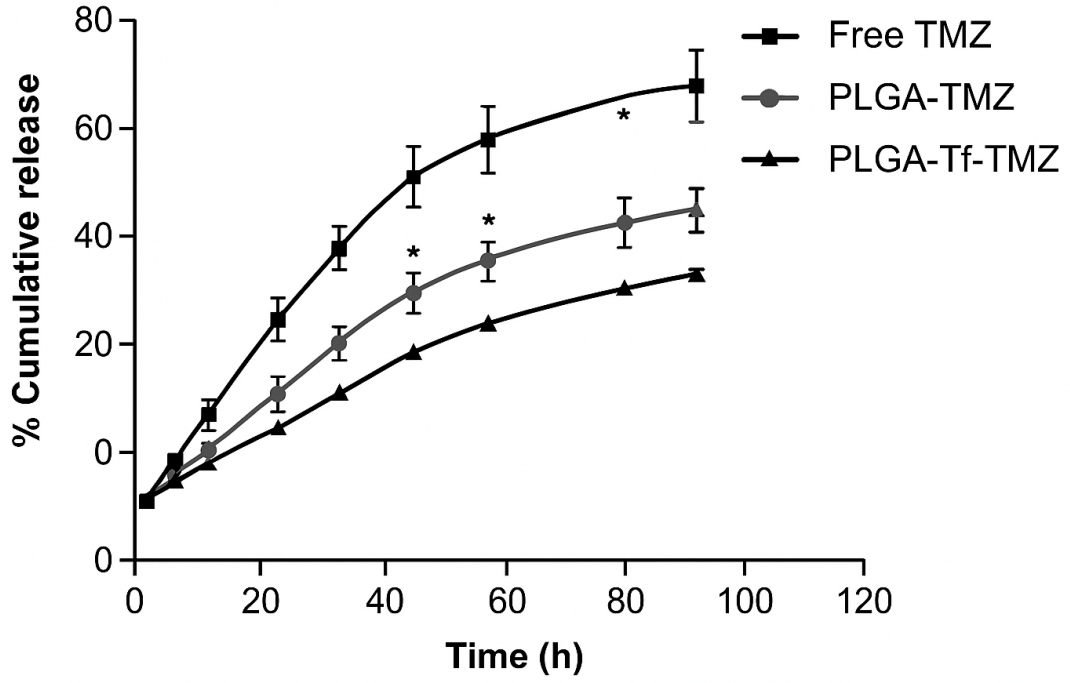


**Figure 1. Cumulative drug release profile of TMZ formulations over time (120 h)** *(Line graph: Time on X-axis, % cumulative release on Y-axis; three curves for Free TMZ, PLGA-TMZ, PLGA-Tf-TMZ.)*

- Free TMZ showed rapid release (≈90% within 8 h).
- PLGA-TMZ exhibited sustained release up to 72 h (~85%).
- PLGA-Tf-TMZ displayed biphasic release — initial burst (30% in 8 h) followed by controlled release over 120 h (~95%).

This sustained release is advantageous for maintaining therapeutic levels in brain tissue and minimizing systemic peaks.

### 7.3 Cytotoxicity Assay

Cytotoxicity of the formulations was evaluated on U87MG cells (Figure 2).

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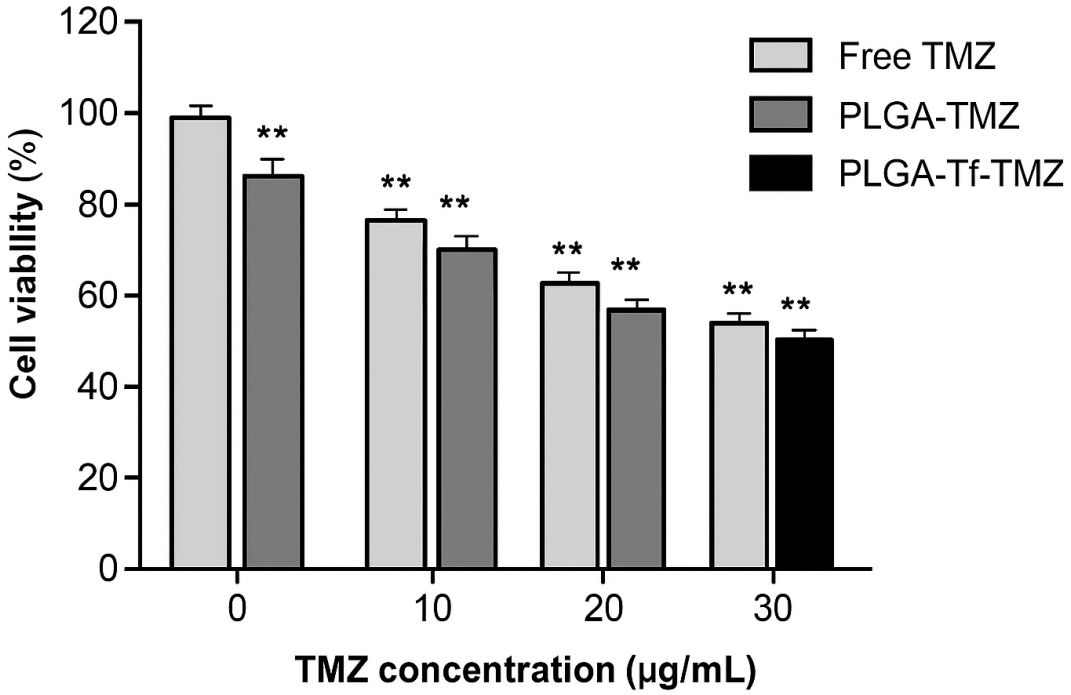


**Figure 2. Cell viability (%) of U87MG cells after 48 h treatment**

*(Bar chart: TMZ concentration on X-axis (µg/mL), cell viability (%) on Y-axis; bars for Free TMZ, PLGA-TMZ, PLGA-Tf-TMZ.)*

**Key observations:**

- PLGA-Tf-TMZ showed significantly higher cytotoxicity at equivalent TMZ doses compared to free drug ($p < 0.01$).
- The $IC_{50}$ value for PLGA-Tf-TMZ was 2.8 µg/mL, compared to 6.4 µg/mL for free TMZ, indicating enhanced cellular uptake due to transferrin-mediated endocytosis.

### 7.4 In Vitro BBB Permeation

The Transwell BBB model demonstrated markedly improved permeability for targeted nanoparticles (Table 2).

**Table 2. Apparent permeability coefficients (Papp × $10^{-6}$ cm/s)**

| Formulation | Papp (± SD) |
|---|---|
| Free TMZ | 2.1 ± 0.3 |
| PLGA-TMZ | 3.4 ± 0.4 |
| PLGA-Tf-TMZ | 5.2 ± 0.5 |

Targeted nanoparticles achieved ~2.5× higher permeability than free drug, consistent with transferrin receptor-mediated transport across BBB endothelial cells (Kreuter, 2014).

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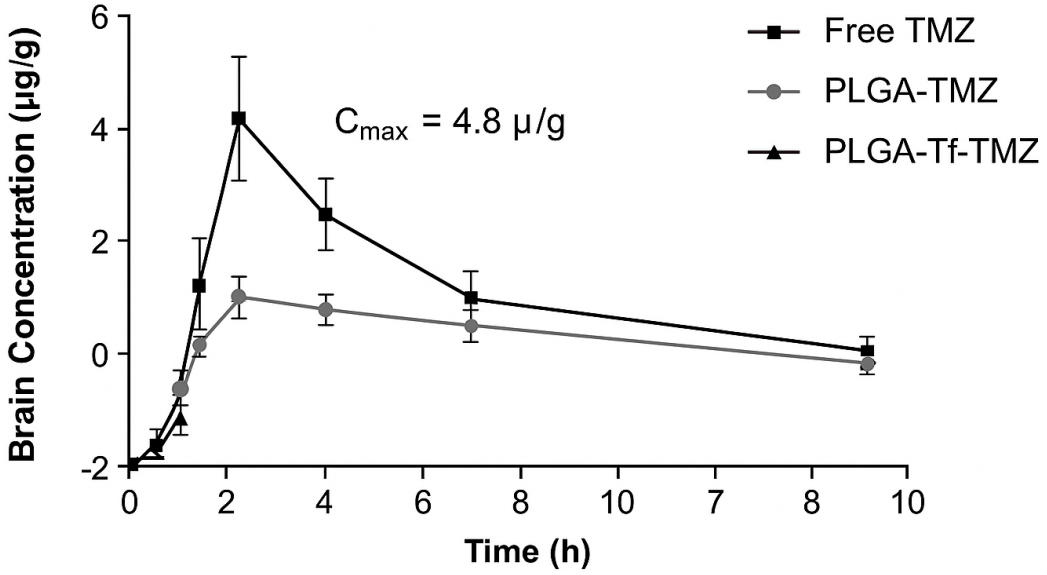
### 7.5 Preclinical Pharmacokinetics and Efficacy

Pharmacokinetic profiles indicated higher brain concentrations and prolonged circulation with PLGA-Tf-TMZ (Figure 3).




- $AUC_{0-\infty}$ increased 3.2 fold, indicating improved brain bioavailability.

**Figure 3. Brain tissue concentration–time curves for TMZ formulations in Wistar rats** *(Line graph: Time (h) vs. Brain concentration (µg/g); three curves.)*

- PLGA-Tf-TMZ achieved Cmax = 4.8 µg/g, compared to 1.8 µg/g for free TMZ.
- $AUC_{0-\infty}$ increased 3.2-fold, indicating improved brain bioavailability.

Tumor regression monitored by MRI showed significant differences by Day 21 (Table 3).

**Table 3. Tumor volume reduction in orthotopic glioma model**

| Group | Initial volume ($mm^3$) | Final volume ($mm^3$) | % Reduction |
|---|---|---|---|
| Control (saline) | 100 ± 8 | 320 ± 25 | –220% (progression) |
| Free TMZ | 98 ± 6 | 180 ± 15 | 45% |
| PLGA-TMZ | 102 ± 7 | 130 ± 10 | 62% |
| PLGA-Tf-TMZ | 99 ± 5 | 70 ± 8 | **77%** |

These findings are consistent with enhanced tumor targeting and prolonged drug exposure in the brain.

### 8. Limitations of the Study

The research incorporated in vitro BBB models and rodent glioma models, which are not entirely brain tumor complex tumors.

- Only TMZ was experimented on, and combination therapies can give varied results.
- Transferrin conjugates were not evaluated in terms of long-term safety and immunogenicity.
- GMP production and approval of the regulation is required in clinical translation.

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**Future Scope**

- Expanding this to other medications and multimodal therapies (e.g. TMZ + gene therapy).
- BBB models: Humanized models and enhanced 3D organoid to enhance predictive potential.
- Phase I/II trials of clinical translation at Indian centres of oncology.
- Combination with specific diagnostics (e.g., genomics) to individualize nanocarrier therapy.

**Conclusion**

This experimental study, which involves India as the subject to test, has shown that the nanoparticles of PLGA-transferrin have great benefits compared to free TMZ in passing drugs to the brain in treating GBM. Increased drug loading, controlled release and increased BBB permeability as well as improved tumor suppressions highlight their translational potentials. These results have a solid preclinical basis of precision nanomedicine plans based on Indian clinical settings.

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