# GENE THERAPY WITH shRNA ANTI-*TERT:* NEW THERAPEUTIC APPROACH FOR GLIOBLASTOMA

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## ANTECEDENTS

### 1. Glioblastoma: unresolved therapeutic challenge

Glioblastoma (GBM) is the most frequent and aggressive cerebral tumor, due to its ability to invade the adjacent brain parenchyma (Image 1).

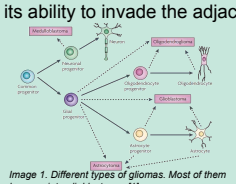

*Image 1. Different types of gliomas. Most of them become into glioblastoma [1].*

Current treatment consists in a surgical resection, followed by radiation and chemotherapy. The median survival for GBM patients is 15 months, because of the high tumor recurrence [1].

The development of new and effective therapeutic strategies for glioblastoma is the future goal.

### 2.Telomeres, telomerase and cancer

Telomere shortening is the first step to tumoral development, following by reactivation of telomerase (Image 2) expression, which allows cellular immortality [2]. 85% of primary human tumors, like GBM, present telomerase activity [3]. New functions of this enzyme are related to tumorigenesis too [4].

For these reasons, and for its specific expression, telomerase is a good therapeutic target against cancer.

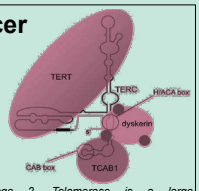


*Image 2. Telomerase is a large ribonucleoprotein complex which adds telomere repeats to chromosome ends. TERT subunit: reverse transcriptase activity; TERC subunit: RNA template [5].*

### 3. Telomerase-based therapies for glioblastoma

*Table 1. Explanation of each telomerase-based therapies and its disadvantages.*

| THERAPY | EXPLANATION | DISADVANTAGES |
|---|---|---|
| Inmunotherapy[4] | Telomerase as antigen | Immune privilege of the brain |
| Telomeric disruption [4] | TERC mutation: telomeric repair inhibition | Complex use *in vivo* / incomplet inhibition of telomerase functions |
| Oncolytic virus [4] | TERT promoter drive expression of suicide genes | Non-reversible treatment |
| Telomerase inhibitor [4] | Imetelstat: RNA TERC inhibition, depletes **glioma stem cells (GSC)** | Systemic administration and temporal action |

### 4. shRNA and telomerase for glioblastoma

New therapeutic strategy for GBM: telomerase gene silencing using shRNA (short hairping RNA, a type of interference RNA), which destroy TERT mRNA, and lentiviral vectors [6], for local administration of this therapeutic transgene shRNA anti-*TERT*. These vectors do not cross the blood-brain barrier.

The treatment is specific of tumoral cells because the biggest part of normal cells does not express telomerase. It can be used to treat glioblastoma directly, or to decrease its recurrence. It is necessary to probe new therapies, like this, in order to solve the therapeutic challenge of glioblastoma.

## HYPOTHESIS

Glioblastoma is an aggressive tumor with high recurrence and current treatments can not remove it at all. For these reasons, new therapeutic approaches are needed. Telomerase is a good target to generate a new therapy for glioblastoma, because its expression is almost specific of tumoral cells, it has other cancer-related functions and GSC can be affected targeting telomerase. Local administration in the brain avoids systemic toxicity and adverse effects in normal cells that express telomerase.

## OBJECTIVES

### 1 To design and to generate a shRNA to produce gene silencing of TERT and validate it *in vitro*, using gene therapy to deliver the therapeutic transgene to target cells

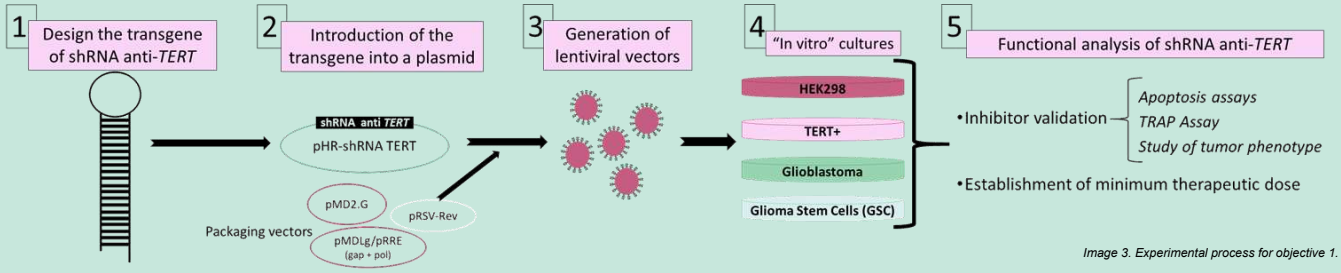


*Image 3. Experimental process for objective 1.*

### 2 To determine the effect of direct administration of lentiviral vectors with shRNA anti-*TERT* into primary glioblastoma tumors using patient-derived tumor xenografts

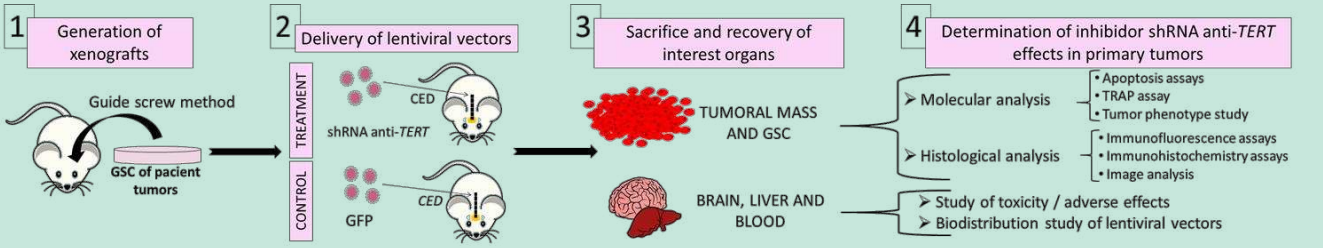


*Image 4. Experimental process for objective 2. In step 2, CED: conventional enhanced delivery.*

### 3 To study the ability of shRNA anti-*TERT* to decrease glioblastoma recurrence by means of the use of patient-derived tumor xenografts treated after surgical resection of tumor

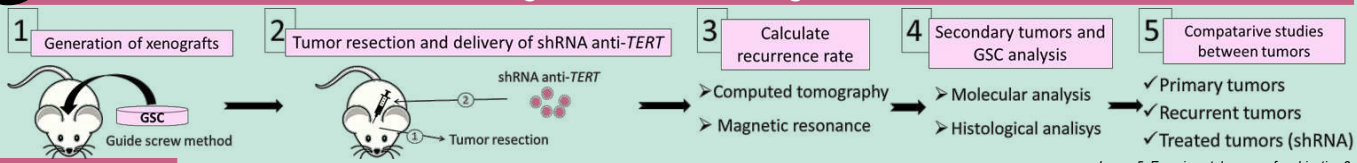


*Image 5. Experimental process for objective 3*

## RESULTS

The complete eradication of glioblastoma using shRNA anti-*TERT* may not be achieved due to complexity and heterogeneity in tumors. Despite this, a high decrease of tumoral cells and GSC is expected, and for that, a high survival in treated xenografts. Adverse effects caused by transgene or lentiviral vector are not expected. If there is not a elevated inhibition of glioblastoma cells, it can be performed a study to probe combined efficiency of shRNA anti-*TERT* with chemotherapy and radiotherapy.

## CONCLUSIONS

The development of new therapeutic strategies for glioblastoma is very important to improve survival of patients and, finally, to remove tumor. Gene therapy using shRNA anti-*TERT* has not been verified yet, but it could be a successful treatment and its research can help us to understand better the complex disease of cancer.

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