TG6002: a Novel Oncolytic and Vectorized Gene-Prodrug Therapy Approach to Treat Glioblastoma

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ABSTRACT

Glioblastoma (GBM) is an incurable disease, which challenges innovation for significant therapeutic progress. TG6002 is a recombinant oncolytic vaccinia virus deleted in two genes (Thymidine Kinase and Ribonucleotide Reductase) and expressing the suicide gene *FCU1* which catalyzes the direct conversion of the nontoxic 5-fluorocytosine (5-FC) into the toxic metabolites 5-fluorouracil (5-FU) and 5-fluorouridine monophosphate (5-FUMP). TG6002 demonstrated strong tumor selectivity and retained full capacity to replicate and lyse human cancer cell lines. The expression of the *FCU1* gene by the recombinant virus provided a targeted chemotherapy within the tumor, with a higher level of efficiency and selectivity than traditional treatment with 5-FU.

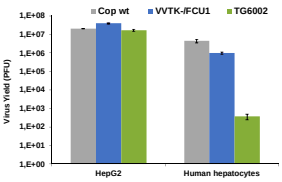
Prior to initiating clinical development, the anti-tumor activity of the TG6002/5-FC combination was investigated, using U-87MG human GBM cell line and glioblastoma stem cells (GSC). The growth of U-87MG subcutaneous tumors implanted in nude mice was inhibited by a single systemic administration of TG6002. In an orthotopic brain tumor model, mice survived significantly longer when treated intravenously by TG6002 alone, and oral 5-FC added a significant survival benefit. GSC retrieved from patients are much less sensitive than U-87MG cells to anti-cancer agents. However, when exposed *in vitro* to TG6002, evidence of virus replication and cell killing was found. In addition, TG6002 yielded a synergistic GSC killing effect when combined with temozolomide in this model, suggesting a potential benefit of this combination in the clinic.

A dose-escalation Phase 1 safety trial of intravenous TG6002 delivery in combination with 5-FC was initiated in patients with recurrent glioblastoma.

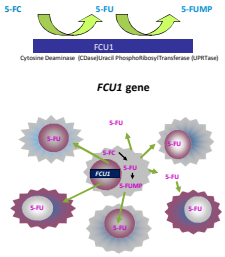
PRODUCT DESCRIPTION

TG6002 = VVTK-RR-/FCU1

- VV: Vaccinia virus strain Copenhagen
- Deletion of *TK* and *RR* genes: attenuated replication in healthy cells
- Expresses *FCU1* gene: combined therapy based on oncolytic activity and targeted chemotherapy

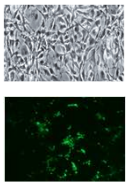


Effect of TK/RR deletions on therapeutic index.
Human hepatocarcinoma HepG2 cells and human hepatocytes were infected by VV wild type (Copwt), VVTK-/FCU1 (single deleted) and TG6002 (double deleted) at 100 pfu. Virus produced after 48h was titrated by plaque assay.

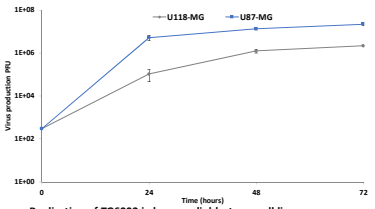


HUMAN GLIOBLASTOMA CELL LINES *IN VITRO*

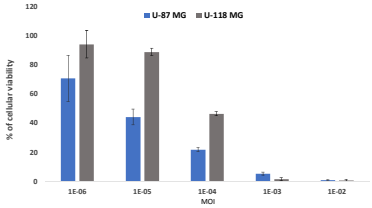
TG6002 showed an efficient infection, replication and oncolytic activity in glioblastoma cell lines



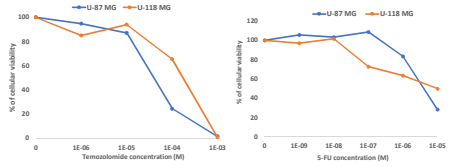
Human glioblastoma cell lines U-87 MG in culture and after infection with TG6002-GFP



Replication of TG6002 in human glioblastoma cell lines.
Human glioblastoma cell lines were infected with TG6002 at MOI 0.001. At 24, 48 and 72h post infection, virus titration was performed by plaque assay.



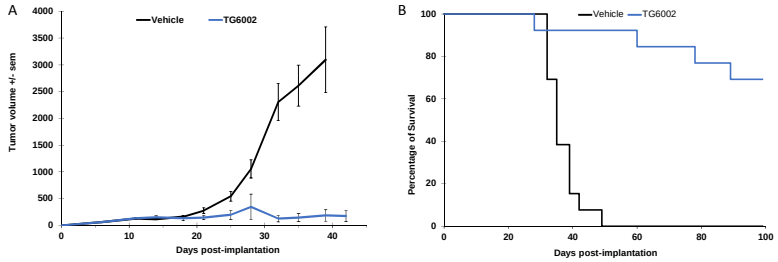
Oncolytic activity of TG6002 in human glioblastoma cell lines.
Human tumor cells were infected at different MOI of TG6002 and cell survival was determined 5 days later.



Sensibility of human glioblastoma cell lines to TMZ or 5-FU.
Human glioblastoma cell lines were cultured with different concentrations of TMZ or 5-FU. After 5 days of treatment, cell survival was determined.

HUMAN GLIOBLASTOMA CELL LINES: SUBCUTANEOUS MODEL

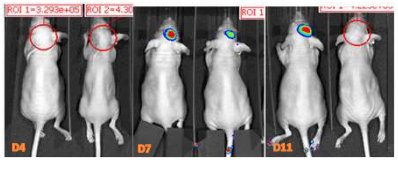
One single I.V. injection of TG6002 resulted in significant antitumor activity



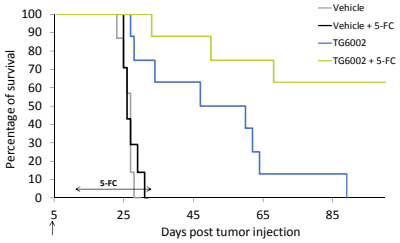
Antitumor activity of TG6002 in subcutaneous models.
TG6002 was injected I.V. in nude mice bearing subcutaneous U-87 MG human tumors. Virus was injected I.V. at 1.10^6 pfu at day 14. A. Tumors were measured in three dimensions and tumor volumes were calculated. B. Survival was based on a sacrifice criteria of 3000 mm^3 of tumor volume .

HUMAN GLIOBLASTOMA CELL LINES: ORTHOTOPIC MODEL

Tumor specific replication of TG6002 (I.V. injection) in human Glioblastoma implanted into the brain leading to a potent antitumor efficiency which was strongly improved by 5-FC treatment



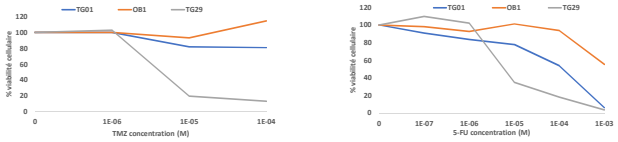
Bioluminescence imaging in orthotopic glioblastoma-bearing mice
For monitoring studies of the distribution of the virus, animals were analyzed for the presence of virus-dependent luciferase activity (TG6002-luciferase). Luminescence images were taken 4, 7 and 11 days after virus injection (I.V.).



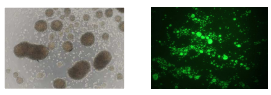
Antitumor activity of TG6002 in an orthotopic human glioblastoma model
TG6002 was injected I.V. at 1.10^6 pfu (arrow) in nude mice bearing orthotopic U-87 MG human tumors. The animals were then treated twice daily with *per os* administrations of saline or 5-FC during three weeks, as indicated by the bar.

HUMAN GLIOBLASTOMA STEM CELLS *IN VITRO*

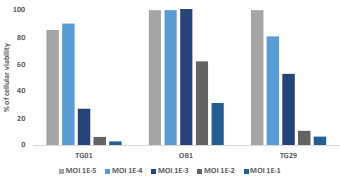
TG6002 displayed potent efficacy in glioblastoma stem cells.



Sensibility of human glioblastoma stem cells cell lines to TMZ or 5-FU
Human glioblastoma stem cells were cultured with different concentration of TMZ or 5-FU. After 5 days of treatment, cell survival was determined.

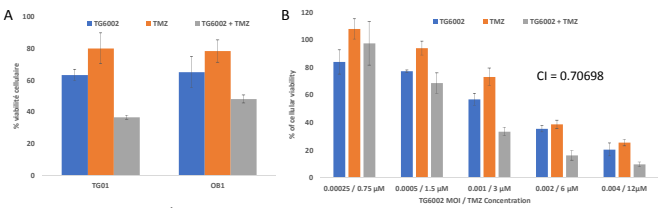


Human glioblastoma stem cells in culture and after infection with TG6002-GFP



Oncolytic activity of TG6002 in human glioblastoma stem cells.
Human tumor cells were infected at different MOI of TG6002 and cell survival was determined 5 days later.

Combination TG6002/TMZ demonstrated enhanced efficiency in GSC compared to single agent therapy.



Combinatorial treatment TG6002/TMZ in human glioblastoma stem cells.
A. TG01 and OB1 were treated with TG6002 or TMZ alone or in combination. The viability was determined by trypan blue exclusion method 5 days following treatment. B. TG29 were treated with a range of the single agents alone or in combination. The viability was determined 5 days following treatment. Combination index value was determined for pharmacologic interaction between TG6002 and TMZ. CI<1 synergism, CI=1 additive, CI>1 antagonism.

CONCLUSION

In human glioblastoma tumor models, after intravenous injection, TG6002 demonstrated **specific replication and high efficacy**. TG6002 showed a **potent antitumor activity** due to its strong oncolytic activity. **Treatment with 5-FC** further provided an **enhanced anti-tumor activity** in orthotopic models.
TG6002 also demonstrated **efficient activity** in several GSC which are known to be resistant to standard radio and chemotherapies and may be the source of cancer recurrence**.** Moreover, the **combination of TMZ/TG6002 displayed a synergistic killing effect** suggesting a potential benefit of this combination in the clinic.
Following these promising preclinical results, a **dose-escalation Phase 1 safety trial of intravenous TG6002 delivery** in combination with 5-FC was initiated in **patients with recurrent glioblastoma** (NCT03294486).