Oncolytic vaccinia virus TG6002 demonstrates potent efficiency in pancreatic tumor alone and in combination with standard chemotherapies

J.Foloppe, C.Pichon, J.Kempf, I.Farine, N.Sfrontato and P.Erbs

Transgene SA, 400 Boulevard Gonthier d'Andernach, Parc d'Innovation, 67405 Illkirch Graffenstaden, France

ABSTRACT

Pancreatic cancer is the fourth leading cause of cancer death in Western countries, with the lowest 1-year survival rate among commonly diagnosed cancers. Surgery remains the single curative option for pancreatic cancer, as few as 20–25 % of patients have resectable disease. Gemcitabine- and 5-fluorouracil (5-FU)-based chemotherapy is the standard treatment for pancreatic cancer following resection.

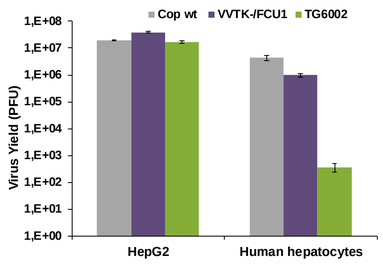
Oncolytic viruses are novel anticancer agents that specifically and effectively kill cancer cells and offers a new strategy to treat pancreatic cancer patient. TG6002 is a recombinant oncolytic vaccinia virus deleted in two genes (Thymidine Kinase and Ribonucleotide Reductase) and expressing the suicide gene *FCU1*. *FCU1* gene encodes a bifunctional chimeric protein that efficiently catalyses 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 potent and significant tumor regression after systemic injection, which is improved in combination with 5-FC administration in many models with weak toxicity.

TG6002 has been evaluated in numerous human pancreatic tumor cell lines *in vitro* where it demonstrated potent replication and oncolytic activity. Moreover, in preclinical studies, TG6002 showed strong efficiency in subcutaneous and orthotopic model after intravenous injection alone and in combination with standard chemotherapy agents.

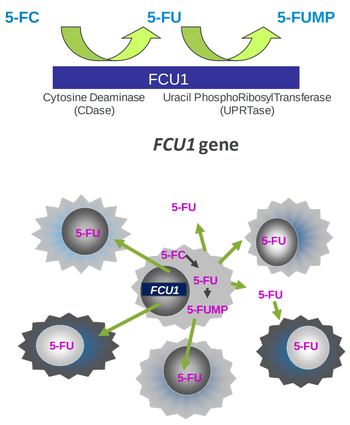
ABOUT TG6002

TG6002 = VVTK-RR-/FCU1

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

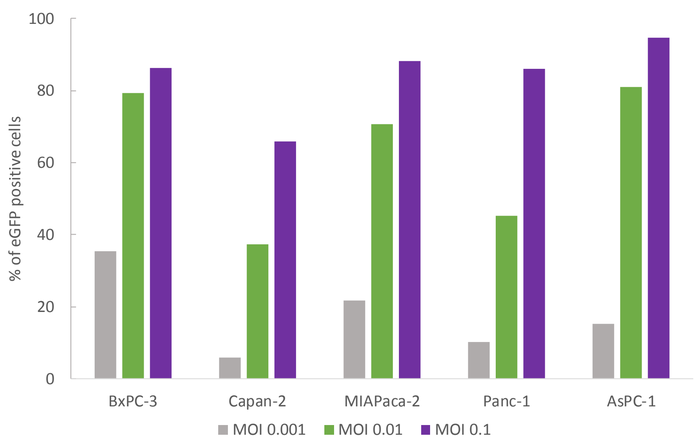


Virus production of the different VV in tumor cells and primary normal cells. Human hepatocarcinoma HepG2 cells and human hepatocytes were infected by VV wild type (Copwt), VVTK-/FCU1 (single deleted) and TG6002 (double deleted) at 100 pfu. Virus produced after 48 h was titrated by plaque assay.

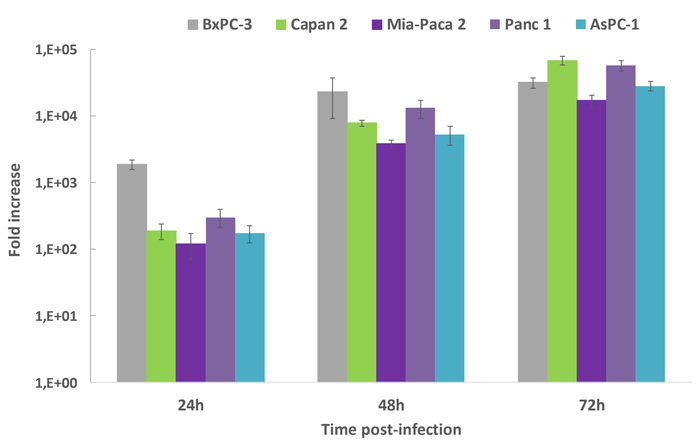


RESULTS *in vitro*

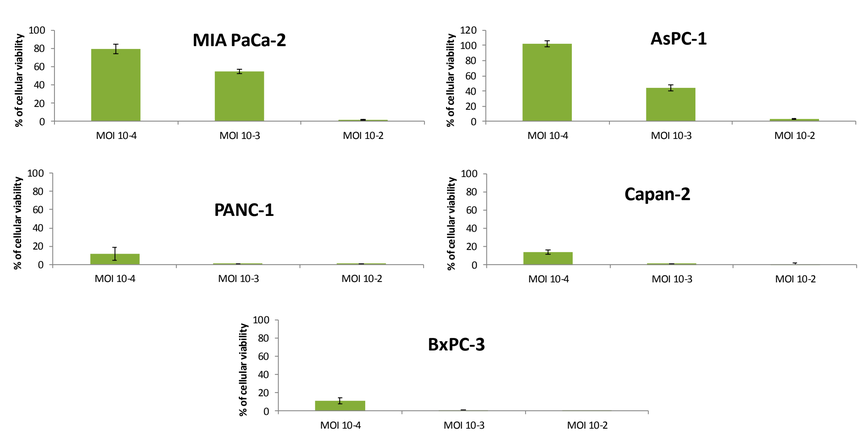
TG6002 showed an efficient infection, replication and oncolytic activity in several pancreatic tumor cell lines



Infectivity of TG6002 in a panel of human pancreatic tumor cells.
Human pancreatic tumor cells were infected with VVTK-RR-/eGFP at MOI 0.001 to 0.01. At 16h post infection, flow cytometry analysis was performed.



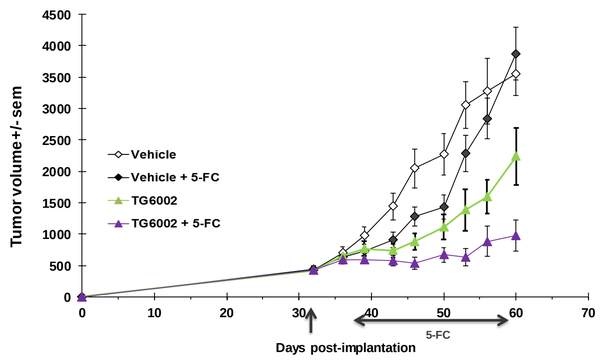
Replication of TG6002 in a panel of human pancreatic tumor cells.
Human pancratic tumor cells were infected with TG6002 at MOI 0.001. At 72h post infection, virus titration was performed by plaque assay.



Oncolytic activity of TG6002 in a panel of human pancreatic tumor cells.
Human tumor cells were infected with a small amount of virus (MOI 0.01, 0.001 and 0.0001) and cell survival was determined 5 days later.

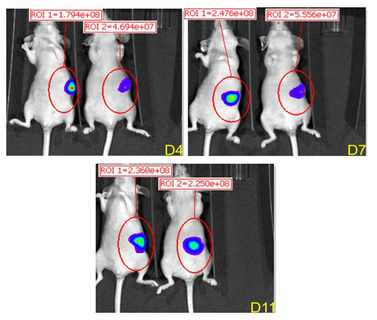
RESULTS *in vivo*

TG6002 displayed potent antitumor activity after one single I.V. injection which was improved by 5-FC treatment in pancreatic tumor model

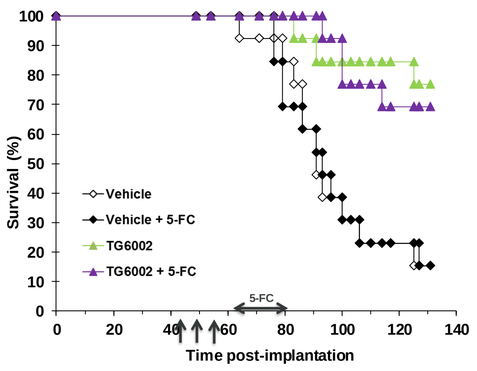


Antitumor activity of TG6002 in subcutaneous models.
TG6002 was injected I.V. in nude mice bearing subcutaneous Mia-PACA-2 human tumors. Virus was injected I.V. at 1.10^7 pfu. The animals were then treated twice daily with *per os* administrations of saline or 5-FC during three weeks, as indicated by the bar. Tumors were measured in three dimensions and tumor volumes were calculated.

Tumor specific replication of TG6002 (systemic injection) in human tumors implanted into the pancreas leading to a potent antitumor efficiency.



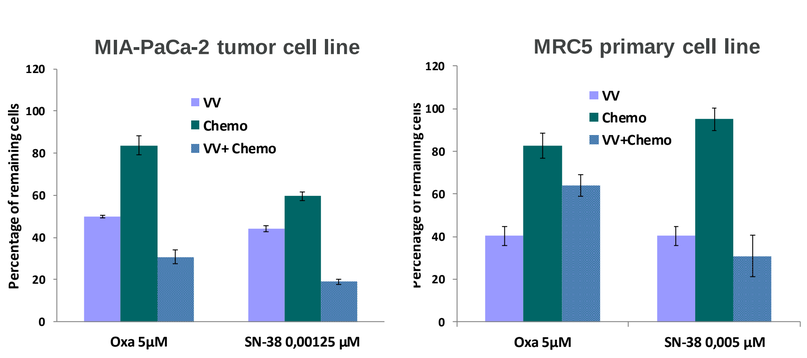
Bioluminescence imaging in orthotopic pancreatic tumor-bearing mice
For monitoring studies of the distribution of the virus, animals were analyzed for the presence of virus-dependent luciferase activity (TG6002-luciferase). Luminescence images were taken 4, 7 and 11 days after virus injection (I.P.).



Antitumor activity of TG6002 in an orthotopic human pancreatic tumor model.

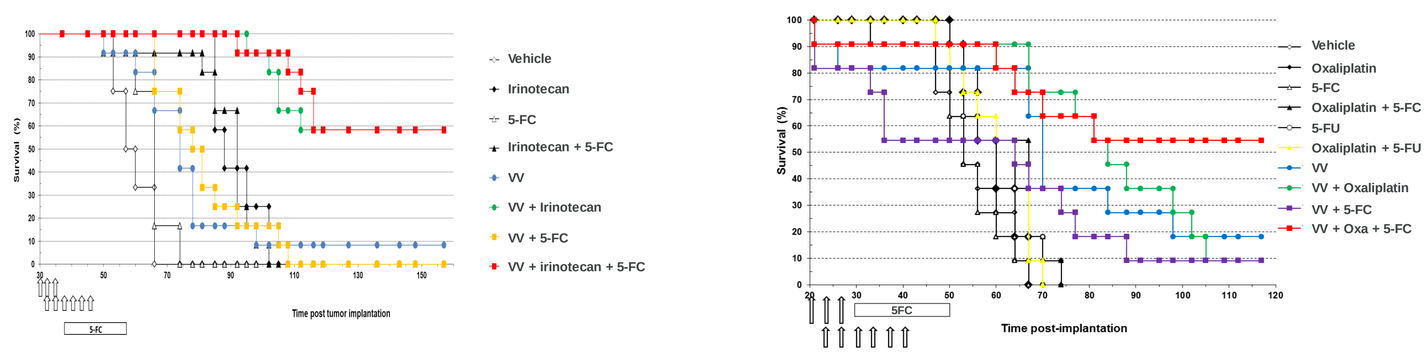
RESULTS combinations

Combination TG6002 with Oxa or SN-38 (active metabolite of Irinotecan) demonstrated enhanced efficiency in cancer cell lines compared to single agent therapy. These synergies were not observed using a primary cell line.



Combination treatment in tumoral and normal cell lines.
Mia-PaCa-2 and MRC-5 were treated with SN38, Oxa or TG6002 alone or in combination. The viability was determined by trypan blue exclusion method (100 % of viability corresponded to non-treated cells)

Three IV injections of TG6002 resulted in a slight improvement in term of survival compared to controls (p<0.05). Irinotecan showed a strong effect which was widely enhanced by TG6002 (±5-FC). In oxaliplatine combination experiment, the greatest benefit in terms of survival has been obtained with the tri-therapy group (TG6002 + Oxa + 5-FC).



Antitumor activity of the combination of TG6002 and irinotecan or oxaliplatin in subcutaneous models.
VV was injected I.V. in nude mice bearing subcutaneous pancreatic MIA-PaCa-2 human tumors. Virus was injected three times at $1x10^6$ pfu as indicated by the first line of arrows. The animals were then treated by Irinotecan twice per week (33 mg/kg/day) or twice a week by oxaliplatin (2.5 mg/kg/day) as indicated by the second line of arrows,. The mice were also treated twice daily with *per os* administrations of saline or 5-FC (200 mg/kg/day), as indicated by the bar. Tumors were measured in three dimensions and Survival was evaluated for the mice bearing MIA-PaCa-2 with a sacrifice criteria of 3000 mm^3 of tumor volume.

CONCLUSION

TG6002 infects, replicates and kills a panel of pancreatic tumor cell lines. In subcutaneous human tumor models, after intravenous injection, TG6002 shows antitumor activity which is improved by 5-FC treatment or combination with oxaliplatine and irinotecan. Moreover, TG6002 displays specific replication and high efficacy in relevant orthotopic model.

These results demonstrate a strong potential of TG6002 in human pancreatic cancer alone or in combination with standard chemotherapeutic agents towards clinical development.