Oncolytic Virotherapy: Harnessing Nature to Treat High-Grade Gliomas and Metastatic Cancer

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Background

Cancers like glioblastoma remain unmanageable for most patients and even with the current optimal treatment offered, survival rates are far too low. Fortunately, several preclinical and clinical studies indicate that oncolytic viruses have the potential to effectively eradicate cancers. Data from one such clinical trial, DNX-240, a recently completed first-in-human Phase I clinical trial using Delta-24-RGD to treat recurrent glioblastoma (NCT00805376) demonstrated that the oncolytic virus successfully excites an antitumor immune response.The oncolytic adenovirus Delta-24-RGD includes a deletion in the E1A region that confers tumor-selective infectivity and an RGD peptide motif insertion that improves infective power.

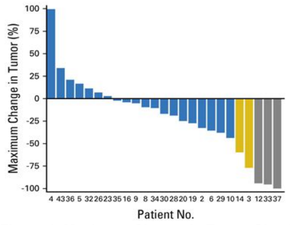


Figure 1. Maximum Change in Tumor Size Among Recurrent Malignant Glioma Patients Treated with 1 Dose of Delta-24-RGD (Lang e/ al., JCO, 2018)
For the majority of patients, the size of their tumors decreased markedly.

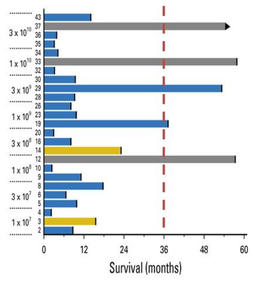


Figure 2. Patient Survival in Months According to Treatment Dose
5 patients survived past 3 years

Hypothesis

We hypothesize that the anti-tumor effect might be amplified by the expression of positive immune checkpoints.

To test this, we incorporated a costimulatory ligand (OX40L) after the fiber region to maximize T-cell activity, which generated our new oncolytic virus Delta-24-RGDOX-DH.

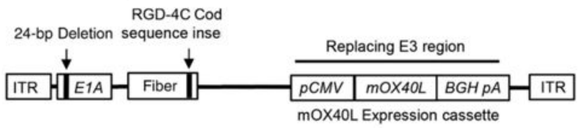
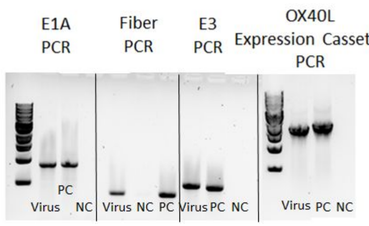


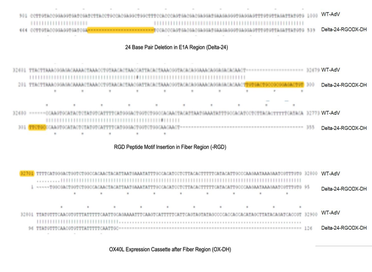
Figure 1. Schematic Illustration of Intended Modifications to the Delta-24-RGDOX-DH viral genome
Delta-24-RGDOX-DH's modifications to the adenovirus genome are: 1. a 24bp deletion in E1A region to allow tumor-selective infectivity, 2. an RGD-4C peptide motif insertion in the Fiber region to boost infectivity of cancer cells. 3. the addition of the sequence coding for the costimulatory ligand OX40 after the Fiber region to prompt the immune system to target infected cancer cells

Methods

The genome of Delta-24-RGDOX-DH was amplified by PCR and sequenced to ensure the viral construct incorporated the appropriate genetic modifications. Next, the expression of murine OX40L in the membrane of infected cells was assessed using western blot and flow cytometry and the expression of other viral proteins by infected cells was analyzed by western blotting. The presence of immune cell populations in tumor-bearing mice was measured between treatment groups with flow cytometry. Afterwards, the oncolytic effects of oncolytic viruses were tested by infecting A549 human lung carcinoma cells and measuring cell counts over time. Then, studies of mice were conducted to compare the therapeutic effect of viruses on 4T1-derived breast tumors compared to a mock infection.

Results





Figures 3 (Left), 4 (Right). Delta-24-RGDOX-DH Sequences of Note
Amplifying (Figure 2) and sequencing (Figure 3) Delta-24-RGDOX-DH DNA indicates the viral construct integrates every necessary edit to form a functional, immune-stimulating oncolytic virus.

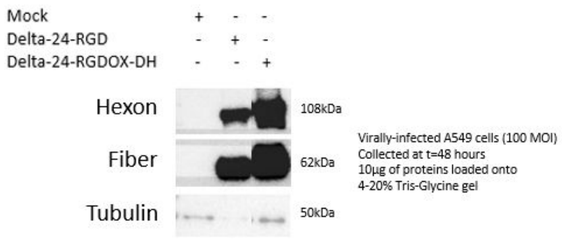


Figure 5. Western Blot Testing Infection-Competency and OX40L Expression on A549 Cell Surface
Only cells infected with an oncolytic virus expressed the proteins Hexon and Fiber which indicate infection-capability. Additionally, the cells infected with the oncolytic viruses that incorporate a costimulatory ligand sequence expressed OX40L on their cell surface.

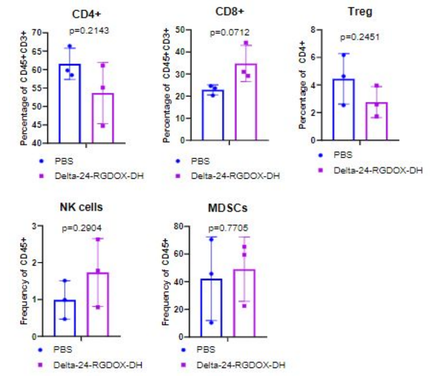


Figure 7. With the immune cell population analysis in the tumor, there is a trend toward an activation of anti-tumor immunity with an increase in CD8+ T cells, Natural Killer cells, and Myeloid-Derived Suppressor Cells, and a decrease in CD4+ T cells and Regulatory T cells.

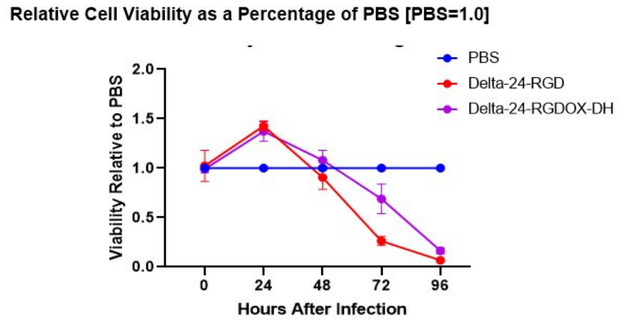


Figure 8. Cell Viability Assay
There was an 85% reduction in cell viability by Day 4 in cells infected with Delta-24-RGDOX-DH compared to cells infected with PBS.
(2-way ANOVA, p=0.0015)

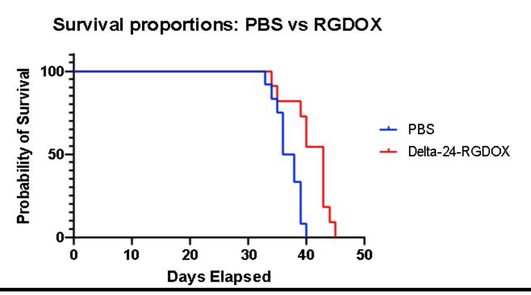


Figure 9 (right). Kaplan-Meier Graph Depicting Survival in Mice with Breast Cancer Treated with Virus vs. Mock Virus
Shows that mice treated with Delta-24-RGDOX survived significantly longer than the mock treatment mice (p=0.0070).

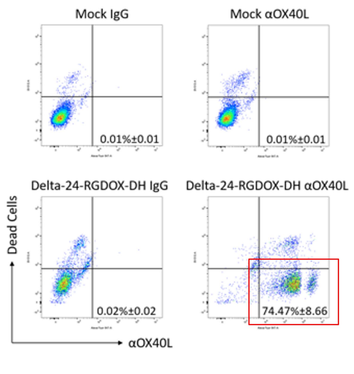


Figure 6. Flow Cytometry of Infected A549 Cells Showing Appropriate Expression of OX40L on Cell Surface
Virally-infected A549 lung carcinoma cells (5MOI) collected at 48 hours present OX40L only once infected with Delta-24-RGDOX-DH

Conclusions

- The Delta-24-RGDOX-DH genome had all the desired genomic alterations that give it the ability to selectively infiltrate and lyse cancer cells, demonstrating reliability in production and utility.
- Experiments showed that Delta-24-RGDOX-DH produced a substantial infection *in vitro* and *in vivo* with effective cancer cell lysis and increased activation of the immune system in tumor-bearing mice.
- Experiments also showed the modified virus produces the necessary costimulatory ligand to maximize infection and reinvigorate immune lymphocytes.
- Delta-24-RGDOX-DH shows promising signs of efficacy as an anti-cancer therapy, so our data should propel the development of clinical trials with Delta-24-RGDOX-DH for patients with high-grade glioma, or other aggressive types of cancer.

Acknowledgements

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