Local and Abscopal Effects in Oncolytic Virotherapy are boosted by Immune Checkpoint Blockade, Immunogenic Chemotherapy, or IFNAR blockade.

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**ABSTRACT**

Although the clinical efficacy of oncolytic viruses has been demonstrated for local treatment, the ability to induce immune-mediated regression of distant metastases is still poorly documented. We here report that an engineered oncolytic Vaccinia Virus, VVwtTKRR-FCU1, is able to induce an immunogenic cell death and thus to generate a systemic immune response. Effect on tumor growth and survival is largely driven by CD8+ T-cells, and we could demonstrate that the immune effect in the tumor could be reprogrammed towards a higher ratio of effector T-cells to regulatory CD4+ T-cells. The key role of the type 1-IFN pathway in oncolytic virotherapy was also highlighted, and we could show a strong abscopal response in *Itlnar* tumors. In this model, the single administration of the virus directly into the tumors, on one flank, led to a regression in the contralateral flank (i.e. opposite to the virus injection site). Moreover, we observed that these effects were further enhanced when the oncolytic treatment is combined with either immunogenic chemotherapy such as oxaliplatin, or with immune checkpoint blockers (ICB) such as anti-PD-1 or anti-CTLA-4. Altogether, these data suggest that local oncolytic virotherapy combined with ICB would best benefit patients harboring tumors altered in IFNAR signaling.

**Vaccinia Virus : VVwtTKRR-FCU1**

- Vaccinia virus is a double strand DNA virus that replicates strictly in cytoplasm : no risk of nuclear integration
- Large DNA insertions are possible (up to 25 kb) as several expression cassettes: enzymes, cytokines, antibodies, ... have been successfully vectorized
- Western Reserve strain: adapted to murine cell replication used as surrogate oncolytic vaccinia virus for in vivo preclinical studies
- Thymidine kinase (TK) and Ribonuclease Reductase (RR) double deleted restrict replication of vaccinia virus to proliferative cells (tumor cells): safer than WT vaccinia virus

**RESULTS**

Figure 1. WR-induced abscopal response in syngenic tumor models

(A) C57BL/6 mice were implanted with 8 × 10^4 MCA205 sarcoma cells. When tumors reached approximately 30 mm³, mice were intratumorally treated with 10^7 pfu WR or control buffer on day 0 and day 3. Mice were monitored daily for survival and in B). Tumor sizes in the treated (B) or untreated (C) are shown as means ± SEM. *** p < 0.001 by log-rank Mantel-Cox test in (B) and (D) and log-rank Mantel-Cox test in (A) and (C)

(B) C57BL/6 mice were implanted with 8 × 10^6 B16F10 melanoma cells. Mice were treated with intravenous injection of 2.5 mg/kg anti-CD8 mAb at day 0 and day 3, and WR was inoculated i.t. at day 0. Tumor sizes are shown as means ± SEM. *** p < 0.001 by log-rank Mantel-Cox test in (B) and (D) and log-rank Mantel-Cox test in (A) and (C)

(C) BALB/c mice were implanted with 8 × 10^6 CT26 tumor cells. When tumors reached approximately 20 mm³, mice were intratumorally treated with 10^7 pfu WR or with control buffer on day 0 and day 3. Tumor sizes are shown as means ± SEM. *** p < 0.001 by log-rank Mantel-Cox test in (B) and (D) and log-rank Mantel-Cox test in (A) and (C)

Figure 2. The antitumor activity of WR is T cell-dependent and is associated with immunogenic cell death

(A) C57BL/6 mice were implanted with MCA205 cells and treated with anti-CD4 or anti-CD8 mAbs 4 days prior to start of treatment with WR. Mice were therapeutically treated with 2 i.t. injections of 10^7 pfu WR or control buffer on day 0 and day 3. Tumor sizes are shown as means ± SEM. *** p < 0.001 and ** p < 0.01 by log-rank Mantel-Cox test

(B) C57BL/6 mice with established MCA205 tumors were intratumorally administered with 10^7 pfu WR or control buffer on day 0 and day 3. Tumor sizes are shown as means ± SEM. *** p < 0.001 and ** p < 0.01 by log-rank Mantel-Cox test

(C) MCA205 WT sarcoma were implanted in C57BL/6 mice and WR was inoculated at 10^7 pfu/50µL (it) at day 1 and day 3. Serum were harvested on day 1, 3, 5, 7, and 11 and IFN-α levels were quantified by ProcartaPlex® Multiplex Immunoassay following manufacturer’s instructions. Each bar represents the mean of 5 mice/group. (D) Ifnar-/- mice were intratumorally treated with 10^7 pfu WR on day 0 and day 3 and with 100 µg/mouse anti-PD-1 mAb on day 0, 3, and 6. Results are representative of 3 independent experiments comparing 0.4 µg/mg. *** p < 0.001, ** p < 0.01, * p < 0.05 by log-rank Mantel-Cox test

Figure 3. The antitumor activity of WR is determined by IFNAR signaling

(A) C57BL/6 mice were implanted with 8 × 10^4 tk-MAC205 tumor cells. When tumors reached approximately 50 mm³, mice were intratumorally treated with 10^7 pfu WR or control buffer on day 0 and day 3, and tumor growth was subsequently monitored. Tumor sizes are depicted. (B) C57BL/6 mice with established MAC205 tumors were intratumorally administered with 10^7 pfu WR or control buffer on day 0 and day 3. HMGB1 data depict the mean. SEM. *** p < 0.001 and ** p < 0.01 by Student’s t-test followed by Dunn’s post test

Figure 4. Characterization of tumor-infiltrating cells following intratumoral WR treatment

(A) BALB/c mice were implanted with 8 × 10^6 CT26 tumor cells. When tumors reached approximately 40 mm³, mice were intratumorally treated with 10^7 pfu WR on day 0 and day 3. At day 6, mice were injected with 10 mg/kg/mouse oxaliplatin. (B) BALB/c mice with established CT26 tumors were intratumorally administered with 10^7 pfu WR or with control buffer on day 0 and day 3. Tumor sizes are shown as means ± SEM. *** p < 0.001 by log-rank Mantel-Cox test in (B) and (D) and log-rank Mantel-Cox test in (A) and (C)

Figure 5. Combination of WR with chemotherapy or immune checkpoint blockers increases therapeutic activity

(A) C57BL/6 mice were implanted with 8 × 10^6 CT26 tumor cells. When tumors reached approximately 40 mm³, mice were intratumorally treated with 10^7 pfu WR on day 0 and day 3. At day 6, mice were injected with 10 mg/kg mouse oxaliplatin. Mice tumor sizes are shown. (B) C57BL/6 mice with established MAC205 tumors were intratumorally administered with 10^7 pfu WR or with control buffer on day 0 and day 3. Tumor sizes are shown as means ± SEM. *** p < 0.001 by log-rank Mantel-Cox test in (B) and (D) and log-rank Mantel-Cox test in (A) and (C)

Figure 6. Combination of WR with chemotherapy or immune checkpoint blockers increases therapeutic activity

(A) C57BL/6 mice were implanted with 8 × 10^6 tk-MAC205 tumor cells. When tumors reached approximately 50 mm³, mice were intratumorally treated with 10^7 pfu WR on day 0 and day 3. Tumor sizes are shown as means ± SEM. *** p < 0.001 by Student’s t-test followed by Dunn’s post test

**CONCLUSIONS**

- VVwt-TKRR-FCU1, a surrogate model for the preclinical study of TG6002, a first-in-class oncolytic virus, induce immunogenic cell death and generate a systemic immune response
- WR is able to reprogram immune cell infiltrate within the tumor microenvironment towards a higher ratio of cytotoxic T cells to regulatory T cells
- Oncolytic Virus alone or in combination with oxaliplatin or immune checkpoint blockers, produces abscopal effects on distant untreated tumors, particularly when the treated tumor displays attenuated type I IFN signaling
- These preclinical data further strengthen the preclinical data package of Transgene’s most advanced next generation oncolytic virus TG6002
- TG6002 is due to enter the clinic in H1 2017 in patients with recurrent glioblastoma