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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, $VV_{WR}TK^{-}RR^{-}$-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 cell infiltrate 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 *Ifnar*$^{-/-}$ 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 : $VV_{WR}TK^{-}RR^{-}$-FCU1

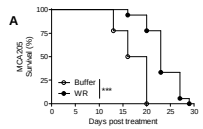
- Vaccinia virus is a double strand DNA virus that replicates strictly in cytoplasm : no risk of nuclear integration
- Large DNA insertions are possible (up to 25 kb) as several expression cassettes: enzymes, cytokines, antibodies, … have been successfully vectorized
- Western Reserve strain: adapted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

CONCLUSIONS

- VV_{WR}-$TK^{-}RR^{-}$-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
- VV 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 check point 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

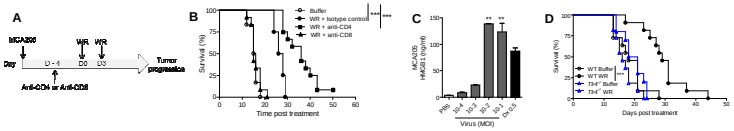
RESULTS

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



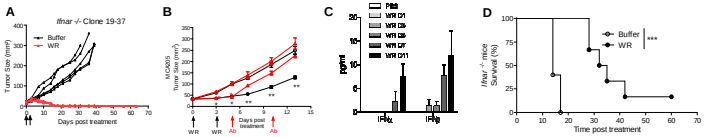
(A) C57BL/6 mice were implanted with 8 × 10^5 MCA205 tumor cells. When tumors reached approximately 40 mm², mice were intra-tumorally injected with 10^7 pfu WR, or with control buffer, on day 0 and day 3. Percentages of surviving mice are depicted. (B) BALB/c mice were implanted with 8 × 10^5 CT26 tumor cells, and treated as in A with WR, or control buffer, on day 0 and day 3. Percentages of surviving mice are shown. Data are representative of 3 independent experiments in A and 4 representative experiments in B. *** p < 0.001 by log-rank Mantel-Cox test

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



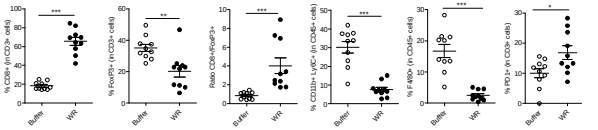
(A) C57BL/6 mice were implanted with MCA205 cells and treated with anti-CD4 or anti-CD8 mAbs 4 days prior to start of treatment with WR. Mice were therapeutically treated with 2 i.t. injections of 10^7 pfu WR or with control buffer on day 0 and day 3. Percentages of surviving mice are depicted in B. (C) Quantification by ELISA of HMGB1 in the supernatants of MCA205 infected with WR or treated with PBS (control) or doxorubicin (0.5 µM). (D) WT or *Tlr4*$^{-/-}$ BALB/c mice were implanted with 8 × 10^5 CT26 tumor cells. When tumors reached approximately 40 mm², mice were intratumorally treated with 10^7 pfu WR or control buffer on day 0 and day 3. Percentages of surviving mice are shown. Results represent at least 2 independent experiments. HMGB1 data depict the mean ± SEM. *** p < 0.001; ** p < 0.01 by Kruskal-Wallis test followed by Dunn's post test (C), and log-rank Mantel-Cox test (B and D)

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



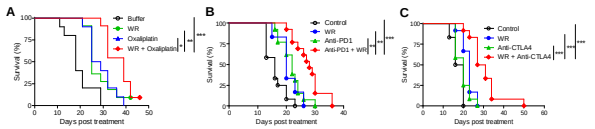
(A) C57BL/6 mice were implanted with 8 × 10^5 of *Ifnar*$^{-/-}$ MCA205 clone 19-37. When tumors reached approximately 40 mm², mice were intratumorally treated with 10^7 pfu WR or with control buffer on day 0 and day 3, and tumor growth was subsequently monitored. Tumor growth is depicted. (B) C57BL/6 mice bearing MCA205 tumors were treated with WR or buffer on day 0 and day 3 as previously described, and received anti-IFNAR1 mAb on days 5 and 10. Tumor size is shown. (C). Serum concentrations of type 1 IFN during local WR infection. 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 (D1, D3). Serum were harvested on day 1, 3, 5, 7, and 11 (as indicated by « D ») and IFNα and IFNβ serum levels were quantified by ProcartaPlex® Multiplex Immunoassay following manufacturer's instructions (ThermoFisher Scientific). Each bar represents the mean of 5 mice/group. (D) Ifnar$^{-/-}$ mice (C57BL/6 background) were implanted with 8 × 10^5 *Ifnar*$^{-/-}$ MCA205 cells (clone 7) and intratumorally treated as before with WR or buffer on day 0 and day 3. Percentages of surviving mice is depicted. Results shown are representative of 2 independent experiments. *** p < 0.001; ** p < 0.01; * p < 0.05 by Kruskal-Wallis test followed by Dunn's post test in B panel and log-rank Mantel-Cox test in D panel

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



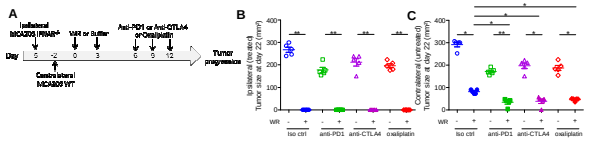
C57BL/6 mice were implanted with 8 × 10^5 MCA205 tumor cells. When tumors reached approximately 40 mm², mice were intratumorally treated with 10^7 pfu WR on day 0 and day 3. Four days after the last injection of WR, tumors were processed for flow cytometry determination of the percentages of $CD8^{+}$ T lymphocytes. The percentage of $FoxP3^{+}$ regulatory T cells (Tregs), intratumoral myeloid-derived suppressor cells (MDSC), the percentage of F4/80^{+} macrophages and the ratio of $CD8^{+}$ T cells to Treg cells is also shown. The expression of the immune checkpoints PD-1 on T lymphocytes was also determined. Results are representative of 2 independent experiments comprising 5-6 mice/group. ***p < 0.001, **p < 0.01, *p < 0.05 by Mann-Whitney test

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



(A) BALB/c mice were implanted with 8 × 10^5 CT26 tumor cells. When tumors reached approximately 40 mm², 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. Means tumor sizes are shown. (B) C57BL/6 mice with established MCA205 tumors were intratumorally administered with 10^7 pfu WR (i.p) on day 0 and 3. At day 6, 9 and 12 mice were treated with 250 µg/mouse anti-PD-1 mAb and their survival was monitored. (C) Similar experimental design to (B) but mice treated with 10^7 pfu WR (i.p) on day 0 and 3 and with 100 µg/mouse (i.p) of anti-CTLA4 mAb on day 6, 9 and 12. Results are representative of 2 independent experiments comprising 5-6 mice/group. *** p < 0.001; ** p < 0.01; * p < 0.05 by log-rank Mantel-Cox test

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



(A) C57BL/6 mice were implanted with 8 × 10^5 MCA205 *Ifnar*$^{-/-}$ cells on one flank and 3 days after with 8 × 10^5 MCA205 WT cells on the opposite flank. When MCA205 *Ifnar*$^{-/-}$ tumors reached approximately 20 mm², mice were intratumorally treated with 10^7 pfu WR on day 0 and day 3. Mice were administrated with 250 µg/mouse anti-PD-1 mAb (at day 6, 9 and 12), with 100 µg/mouse anti-CTLA-4 mAb (at day 6, 9 and 12), or with 10 mg/kg/mouse oxaliplatin (at day 6). Tumor sizes in the ipsilateral (treated,B) or contralateral (untreated, C) are shown as means ± SEM. The experiments have been performed twice with similar results. *** p < 0.001; ** p < 0.01; * p < 0.05 by Kruskal-Wallis test followed by Dunn's post test