TG6050, an oncolytic vaccinia virus armed with interleukin 12 and anti-CTLA4 antibody induces TME remodeling and strong anti-tumoral responses.

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Abstract 694
Poster 14

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BACKGROUND

By replicating specifically into tumor cells, oncolytic vaccinia viruses (VACV) can turn "cold" tumors "hot" while delivering therapeutic payloads into tumor. Thanks to its large genome capacity, multiple cloning loci, and availability of different promoters, VACV is an optimal vector for the design of advanced immunotherapies. Payloads with an established clinical efficacy but limited in terms of tolerability require local delivery. We report here the preclinical characterization of **TG6050**, a **VACV encoding single chain interleukin 12 (IL12) and anti-CTLA4**, targeting multiple cancer indications.

METHODS

TG6050 was generated by insertion of both the single chain IL-12p70, and an anti-CTLA-4 full-length (heavy and light chains independently) into the thymidine kinase (TK) and ribonucleotide reductase (RR) loci, of a triple deleted (ΔJ2R,ΔI4L,ΔM2L) VACV, Copenhagen strain. TG6050 was fully characterized in vitro, i.e. replication in tumor and normal cells, transgenes expression, functionality, and genetic stability. Its preclinical surrogate mTG6050, expressing murine payloads, was used to investigate the in vivo **anti-tumoral activities** in a broad range of **immunocompetent** murine models, as well as its mode of action in the **reprograming of the tumor microenvironment** (TME).

RESULTS

TG6050 displayed the same replicative, oncolytic activity, and genetic stability features as benchmark recombinant vaccinia viruses. IL-12 and anti-CTLA4 were both expressed at high levels, and as functional molecules by a broad panel of reference tumor cell lines.

Local administration of mTG6050 induced **accumulation of transgenes into tumor**, **with low systemic exposure**. Noteworthy, expression of IL-12 did not accelerate the clearance of the viral vector. The combined effects of viral replication, IL-12 and anti-CTLA4 expression translated into **impressive antitumoral activities** in several syngeneic tumor models including immune-resistant ones such as **B16F10 or LLC1**.

Transcriptomic analyses of the TG6050-treated tumors demonstrated a **strong dynamic of infiltration by innate and adaptive immune cells**. IFNγ-ELISpot analysis on splenocytes confirmed induction of **a strong systemic and specific anti-tumoral immune response**.

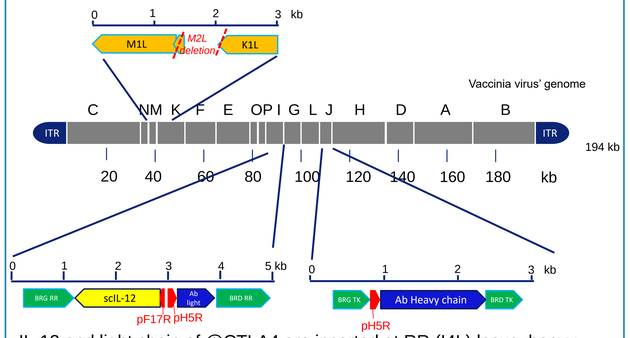
Moreover, the combination of TG6050 with ICI improved tumor regression in several challenging tumor models.

CONCLUSION

TG6050 is a novel oncolytic **VACV designed for multiple routes of administration**, and several tumor indications. Its strong impact on the TME, by massive infiltration of innate and adaptive immune cells, translated into very remarkable therapeutic activities in resistant tumors. This anti-tumor activity was further enhanced by combination with anti-PD-1.

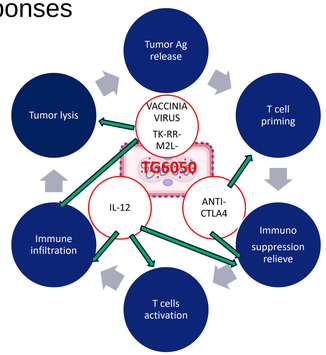
TG6050 DESIGN AND MODE OF ACTION

TG6050 design



IL-12 and light chain of @CTLA4 are inserted at RR (I4L) locus, heavy chain of @CTLA4 is inserted at TK (J2R) locus.
scIL12: single chain IL-12: p40-GS-p35 fusion.
pH5R and pF17R are poxpromoters.

TG6050 putative mode of action on anti-tumor responses



Vaccinia virus replicates and **kill tumor cells**, and induces inflammation and **immune infiltration.**

Released tumor antigens are taken and presented by APC, @CTLA4 frees B7 co-stimulation molecules (in absence of M2L viral gene) for signal 2 and **T cell priming.**

@CTLA4 and IL-12 **relieve immunosuppression** by acting on Treg and myeloid-derived suppressor cells (MDSC).

IL-12 **activate T and NK cells,** that secrete **IFNγ** that in turn induces CXCL9 and **CXCL10 chemokines production.**

Chemokines attract more immune cells that **infiltrate the tumor.**

KEY FINDINGS

- ✓ High expression of **functional IL-12** and **@CTLA4** into the tumor with **low systemic exposure**
- ✓ Unprecedented anti-tumor activity in several murine tumor models
- ✓ Anti-tumor adaptive immune response boosted with profound TME remodeling

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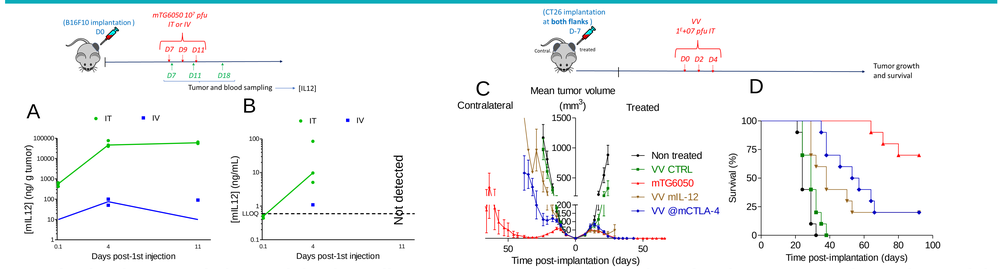
TG6050 induces tumor regression by activating innate and adaptive anti-tumoral immune responses

mTG6050 allows sustained expression of IL-12 into tumor and has anti-tumoral activity



Vectorization into oncolytic vaccinia virus allows tumor restricted expression of active concentrations of IL-12 (A) with minimal systemic exposure (B).

In CT26 two-tumors model, both IL-12 and @CTLA4 are necessary to have optimal regression of contralateral tumor (C) and improved mice survival (D). This activity on distant lesion suggests that a robust systemic antitumoral response has been raised by mTG6050.

TG6050 boosts innate and adaptive anti-tumoral immune responses

mTG6050 treatment increased dramatically the T cell response against both tumor derived peptide and killed tumor cells compared to control virus (VV CTRL).

mTG6050 treatment increased transcriptomic signatures of numerous innate and adaptive immune pathways into the tumor.