Transforming all Tumor Sites: The Power of Systemic Enveloped Virotherapy

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Abstract

Systemic oncolytic virotherapy offers a promising solution for treating both local and metastatic diseases. However, the rapid inactivation of virotherapeutics by the immune system has led to disappointing clinical efficacy. To address this challenge, we developed a cell-based oncolytic virotherapy that successfully targets distant tumor sites without rapid elimination by the host immune system. This therapy utilizes a newly isolated, tumor-selective strain of vaccinia virus that produces high levels of extracellular enveloped virions (EEVs) incorporating human cell-derived proteins/tumor-associated antigens (TAA). This newly developed virotherapy provides enhanced protection against the immune system and achieves durable therapeutic efficacy when administered systemically.

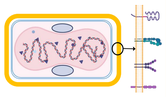
Summary

We have engineered a **novel tumor-selective vaccinia virus strain**, named Redtail (RT), capable of producing a high amount of **enveloped vaccinia viruses (envRTs)** (Resistant to humoral immunity)

We have developed a **new manufacturing process that enriches the EEVs, and maintains integrity of EEV for long-term storage,** making this technology feasible for the first time.

This technology will allow us to **reach every tumor systemically, kill tumor cells, and express any desired protein** within the tumor and change all tumor microenvironments (TME).

Redtail: An Enveloped Vaccinia (EEV) Strain Designed for Systemic Delivery



Enveloped:

***1- High production of* enveloped viruses** is genetically encoded in virus genome.

2- Virus is ***manufactured*** enveloped with a human cell membrane containing **human surface receptors** offering:

- **Protection/immunomodulation**
- ***Targeting/Tumor Homing***

Safety:

1- New **genetically engineered tumor selective Vaccinia virus**

2- Does not integrate into human genome.

3- *Platform has a* ***Safety-switch*** (antiviral, FDA approved)

Multifunctional Human Surface Proteins Expressed in Enveloped Virus

A. Vaccinia virus has 2 main forms:

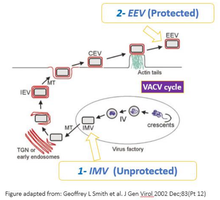
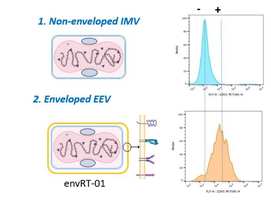


Figure adapted from: Geoffrey L Smith et al. J Gen Virol 2002 Dec;83(Pt 12)

B. Example flow cytometry of viral particles expressing CD55 (DAF)



Enveloped viruses incorporate human surface proteins in their extracellular envelope. Examples: CD55, CD44, CXCR4

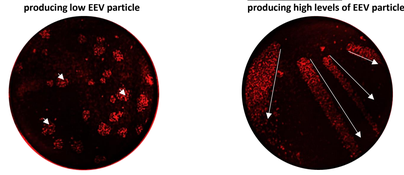
EEV form of envRT expressing multiple human surface proteins.

A. Vaccinia virus exists in two primary forms – intracellular mature virus (IMV) and extracellular enveloped virus (EEV). While IMV is prevalent in most strains, **we selected a new vaccinia virus clone/strain which produces drastically higher levels of EEV**, offering superior systemic delivery, enhanced spread, and antitumor immunity.

B. Flow virometry was employed to analyze the expression of human surface proteins in non-enveloped IMV and enveloped EEV vaccinia virus. The blue histogram depicts non-enveloped IMV with no CD55 expression, whereas envRT (EEV) shows positive CD55 expression, as indicated in the orange histogram. The production of EVVs by envRT allows for the expression of important human proteins that confer protection against human complement, facilitate targeted tumor cell recognition, and enhance homing capabilities for precise therapeutic intervention.

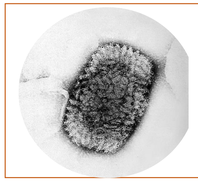
Identifying a Novel Vaccinia Virus Strain with Enhanced EEV Production

Comet assay of two distinct vaccinia viruses



Vaccinia Viruses are genetically engineered to express TurboFP635 (red fluorescence).

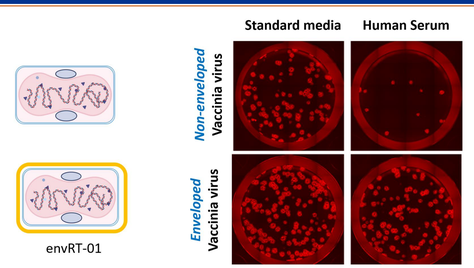
Vaccinia Virus as a Systemic Antitumor Virotherapy and Viral Vector



1- RT Vaccinia virus is a **highly cytolytic virus** - tumor agnostic.
2- Genetically stable. 200kB dsDNA virus.
3- Large insertion capacity (25-45Kb), allowing delivery of therapeutic proteins into the tumor, potentiating antitumor systemic virotherapy efficacy.

- Cytokines.
- Checkpoint inhibitors, agonists, multiple types of therapeutic antibodies.
- Other TME modifiers.

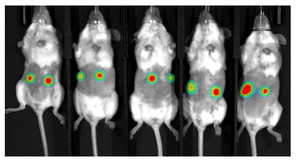
New Manufacturing Process Ensures Second Membrane Integrity



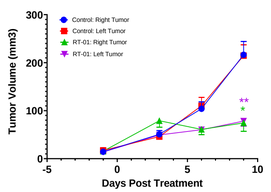
EEV particles but not IMV are resistant to human complement. Unlike IMV, EEV particles exhibit resistance to human complement. Equal quantities of IMV and EEV viral particles were exposed to 20% human serum for 1 hour, followed by viral plaque assay (VPA). As depicted in the fluorescent images from the VPA, only EEV particles, engineered to express TurboFP fluorescence (bottom right), demonstrated resistance to humoral immunity.

envRT Inhibits Lung Cancer In Immunocompetent Mouse Model

Dual subQ tumor bearing lung cancer

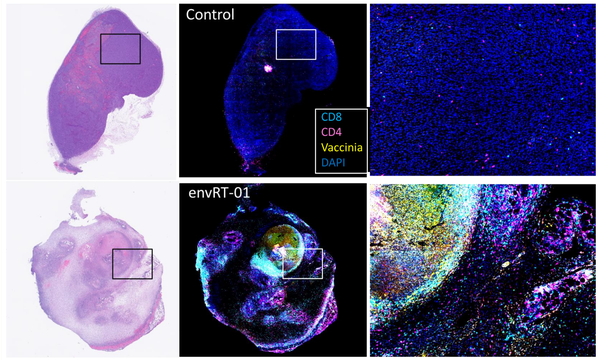


envRT Virus expressing TurboFP635: TurboFP635 is represented as Rainbow signal
- Image was taken 3 days post virus injection

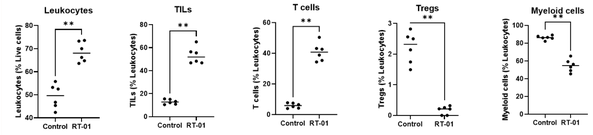


Inhibition of tumor growth by single dose envRT Treatment in LL2 lung syngeneic mouse model. Mice were subcutaneously inoculated with LL2 cells in the abdomen. Once tumors reached 60-100 mm^3, animals were intravenously treated with a single dose of 3.5e6 PFU envRT or buffer control (n=5 per group). Tumor growth was measured twice per week.

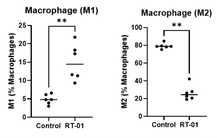
envRT Induces Dramatic Changes in Tumor Immune Microenvironment



Systemic administration of envRT changes TME in lung subQ tumor model (Immunocompetent).
Representative multiplex IHC images of envRT-treated LL2 lung tumors implanted on the side flank show dramatic TME changes with immune cell infiltration to the tumor site 7 days post virus injection. From left to right: H&E, whole section multiplex IHC, and magnified selected area. Control (top line), envRT-01 treated (bottom line).



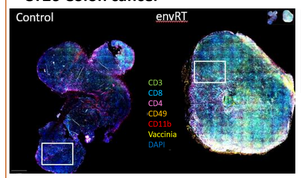
Macrophage subsets

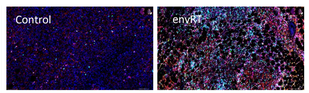


Dramatic changes in TME after envRT administration in lung subQ tumor model (Immunocompetent).
1e6 LL2 cells were implanted on the right flanks of C57BL mice. 5 days post implantation, a single dose of 3.5e6 PFU envRT was intravenously injected. Tumors were collected 7 days post virus injection for TIL analysis (n=5). envRT induced increased leukocyte, TILs, T cells, and decreased Tregs and myeloid cells in the LL2 mouse lung tumors. Macrophage polarization towards M1 from M2 was also observed. Similar results were found in EMT6 (Breast cancer) and CT26 (Colon cancer).

envRT Induces Dramatic Changes in Colon and Breast Cancer TMEs

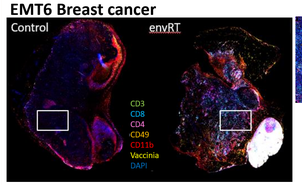
CT26 Colon cancer

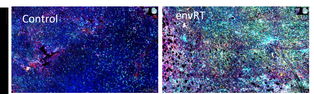




Dramatic changes in TME after envRT administration in CT26 colon cancer model (Immunocompetent).
Representative multiplex IHC image of envRT-treated CT26 colon tumors. 2.5e5 CT26 cells were implanted on the right flanks of Balb/c mice, followed by i.v. treatment of 3.5e6 PFU envRT 5 days post implantation.

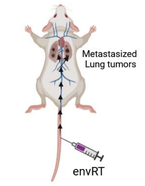
EMT6 Breast cancer



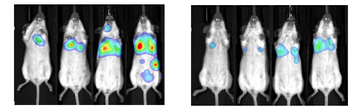


Dramatic changes in TME after envRT administration in EMT6 breast cancer model (Immunocompetent).
Representative multiplex IHC image of envRT-treated EMT6 breast tumors. 1e6 EMT6 cells were implanted on the right flanks of Balb/c mice, followed by i.v. treatment of 3.5e6 PFU envRT 5 days post implantation.

envRT Reduces Lung Tumor Burdens at Metastatic Sites



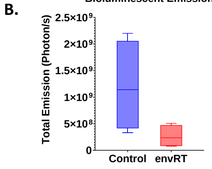
A. Control envRT



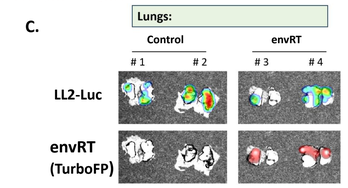
Immunocompetent Mice

envRT Virus expressing TurboFP635: TurboFP635 is represented as Rainbow signal
- Image was taken 6 days post virus injection

B. Bioluminescent Emission

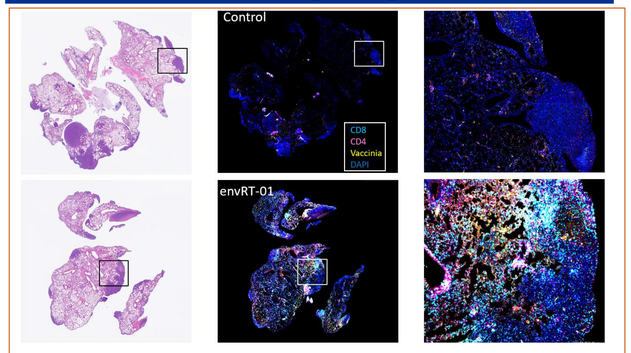


C.



Systemic administration of envRT reduces tumor burdens at lung metastatic sites. To generate experimental lung metastasis, 1e6 lung-metastatic LL2-Luc cells were intravenously administered via the tail vein of C57/B6 mice. Eight days post-inoculation, animals received an i.v. treatment of 5e6 PFU of envRT expressing fluorescent TurboFP protein twice, three days apart. **A.** The bioluminescent image shows reduced tumor burden with envRT treatment 6 days post virus treatment. **B.** The graph shows the total bioluminescent emission of lung-metastatic LL2-Luc tumor signals 6 days post virus treatment. **C**. The ex-vivo imaging indicates that envRT targets metastasized LL2 tumors in the lung.

envRT Induces Dramatic Changes in Lung Metastases TMEs



Systemic administration of envRT changes lung metastases TME. Representative multiplex IHC images of envRT-treated LL2 lung tumors show envRT specifically targeted tumors and drastically induced recruitment of immune CD4 and CD8 cells into the TME. From left to right: H&E, whole section multiplex IHC and magnified selected area. Control (top line), envRT treated (bottom line).

Pipeline and Early Partnership Opportunities

Program	Mode of Action	Target Indication	Discovery/Preclinical	IND-enabling	Phase 1
EnvRT-01s Lead generation	• Tumor killing • TME reprogramming, • Payload undisclosed	Metastatic NSCLC (Lung Cancer)			
EnvRT-02s Combo with Immune cell therapy	• Tumor killing • Prime solid tumors to prepared for combo Tx	Advanced solid tumors (Indication undisclosed)			
EnvRT-03s Novel Payload	• Improved TME reprograming and Killing. • Payload undisclosed	Target indication undisclosed			