Development of a Systemic Enveloped Virotherapy for Targeting Metastatic Sites

Building a multimodal immunotherapy able to deliver IL15-IL15Rα

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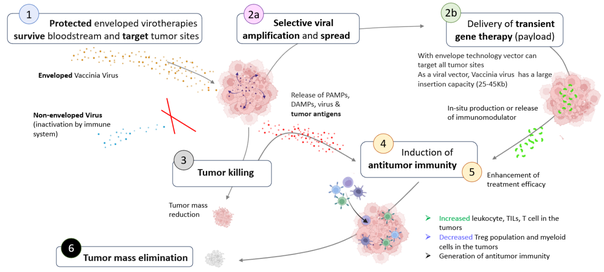
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Abstract

Systemic virotherapies targeting directly all tumors are a promising form of cancer immunotherapy not only due to their tumor lytic characteristics but also because of their potential to transform tumor immune microenvironment, and targeted delivery of virus-encoded therapeutic payloads into tumors. Developing a platform for systemic delivery would provide a significant advantage in treating both primary tumors and any metastatic lesions. However, systemic administration of virotherapies may pose challenges of increased toxicity and reduced efficacy due to rapid virus clearance. In order to overcome these limitations, we have selected and developed a novel enveloped vaccinia virus platform by isolating a complement-resistant strain called Redtail vaccinia virus (RT). We have further engineered the backbone of the isolated strain to achieve tumor specific targeting and high therapeutic efficacy:

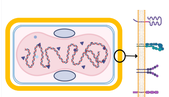
Multifunctional Mechanism of Action of Enveloped RT Virotherapy



Systemic administration targeting all tumors transforms oncolytic virotherapy into a viral vector capable of locally expressing selected transgenes at each tumor site.

IL15-IL15Rα (IL-15 superagonist) transgene would activate the IL-2/IL-15 receptor complex, enhancing the therapeutic effect of the systemic virotherapy.

Redtail (RT): 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**

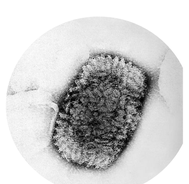
Safety:

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

2- Does not integrate into human genome.

***3-* Available FDA-approved antiviral drugs**

Vaccinia Virus as a Systemic Antitumor Virotherapy and Viral Vector



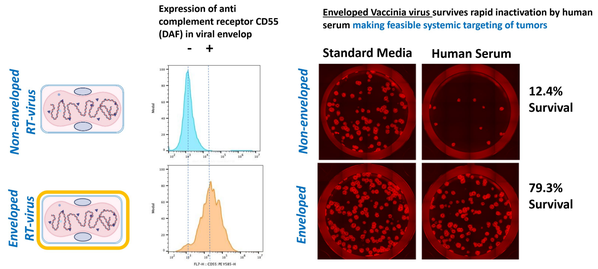
1- RT Vaccinia virus is a **highly cytolytic virus** - tumor agnostic.

2- Genetically stable. 200kb dsDNA virus.

3- Large insertion capacity (25-45Kb), allowing delivery of existing therapeutic proteins into the tumor, potentiating antitumor systemic virotherapy efficacy.

- Cytokines.
- Checkpoint inhibitors, agonists, multiple types of therapeutic antibodies.
- Other tumor microenvironment (TME) modifiers.

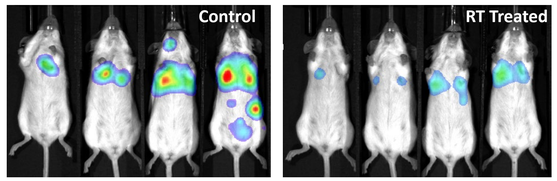
Envelope Maximizes Resistance Against Humoral Immunity

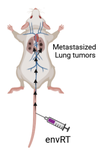


We developed a novel methodology to manufacturing extracellular enveloped vaccinia viruses (EEVs)

EEV particles but not IMV are resistant to human complement. Standard manufacturing processes focus on producing IMV (intracellular mature virus) rather than EEV (extracellular enveloped vaccinia viruses), necessitating a new process to manufacture EEV.
Unlike IMV, EEV particles exhibit resistance to human complement. Equal amounts of IMV and EEV viral particles were exposed to 20% human serum for one hour, followed by a viral plaque assay (VPA). The fluorescent VPA images reveal that only EEV particles engineered to replace TK (thymidine kinase) and express TurboFP fluorescence (bottom right) demonstrated resistance to humoral immunity—designated as the 1st generation.

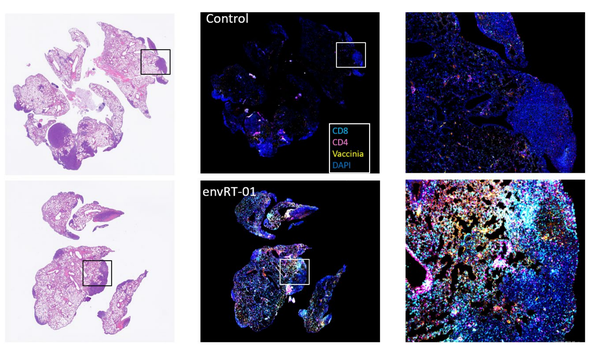
RT Virus Can Effectively Target Metastatic Locations





The systemic delivery of RT virus diminishes tumor loads at lung metastatic sites. C57/B6 mice received 1e6 lung-metastatic LL2-Luc cells through intravenous injection via the tail vein. Eight days after tumor implantation, 5e6 PFU of RT 1st generation was administered intravenously three times, spaced three days apart. The bioluminescent imaging indicates a decrease in tumor burden following RT treatment, observed six days after the virus administration.

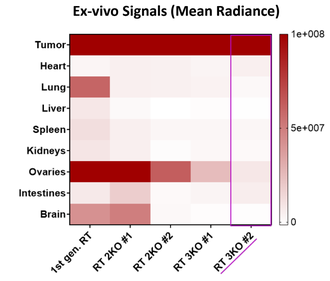
RT Virus Induces Dramatic Changes In Lung Metastasis



Systemic administration of RT induces significant changes in lung metastasis tumor microenvironments (TMEs). Representative multiplex IHC images of LL2-luc lung tumors show RT-treated samples exhibiting precise tumor targeting and robust immune cell recruitment, including CD4+ and CD8+ T cells, 7 days post-treatment with a single dose of 3.5e6 PFU RT 1st generation virus. The images progress from H&E staining (left), to whole-section multiplex immunohistochemistry IHC (middle), and magnified selected areas (right). Control group (top row), RT-treated group (bottom row).

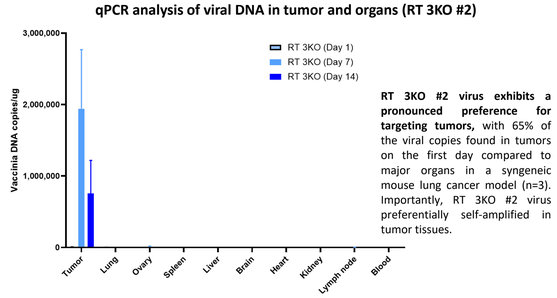
Generation of a Tumor-Selective RT (3KO) Vaccinia Virus

RT 3KO #2 (TK-, A46R-, VGF-) Preferentially Targets And Amplifies In Tumors And Not In Other Major Organs.



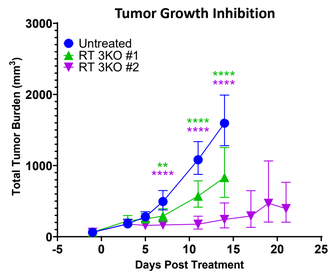
Ex-vivo imaging of major organs after intravenous administration of different engineered (KO) RT viruses (expressing TurboFP).

- RT viruses with double KO (RT 2KO) or triple KO (RT 3KO) reduce off-tumor toxicities in the Non-Small Cell Lung Cancer NSCLC athymic mouse xenograft model.



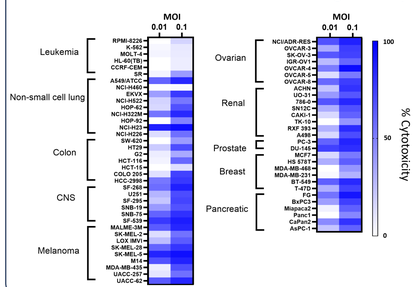
RT 3KO #2 virus exhibits a pronounced preference for targeting tumors, with 65% of the viral copies found in tumors on the first day compared to major organs in a syngeneic mouse lung cancer model (n=3). Importantly, RT 3KO #2 virus preferentially self-amplified in tumor tissues.

RT 3KO #2 Promotes A More Effective And Lasting Therapeutic Response In Syngeneic Mouse Models Of Lung Cancer



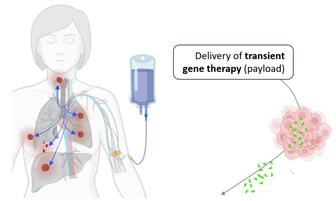
Tumor growth inhibition after administration of different engineered (KO) RT viruses in the syngeneic lung tumor model. Mice were subcutaneously inoculated with LL2 cells, and once tumors reached 60–100 mm³, animals were treated intravenously with a single dose of 5e6 PFU RTs or buffer control (n=5 per group). RT 3KO #2 preferentially targeted tumors, leading to enhanced therapeutic efficacy and improved survival in the C57BL/6 syngeneic lung tumor model.

RT 3KO #2 Induces a Robust and Direct Killing of Multiple Tumor Cell Types



Analysis of tumor cytotoxicity for RT-3KO #2 across over 60 human tumor cell lines using the NCI-60 panel: RT 3KO #2 demonstrated notable and direct cytotoxic effects after 72 hours post-treatment at a lower multiplicity of infection (MOI), successfully killing almost all tumor cell lines linked to different kinds of solid tumors.

The Clinical Potential Of Enhanced RT 3KO Virus For Delivering IL15-IL15Rα To Neoplastic Locations

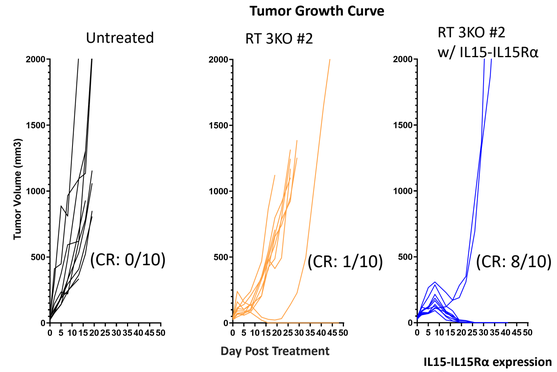


Local production of IL15-IL15Rα

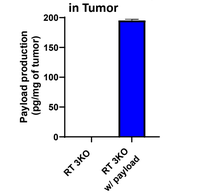
- ✓ Enhanced immune activation
- ✓ Expansion of NK and CD8+ cells
- ✓ Promotion of cytotoxic cytokine release
- ✓ Long-lasting effect by boosting memory CD8+ T cells
- ✓ Clinically proven efficacy

When deliver as transgene allows: In-situ production and release in all tumor microenvironments (TME)

IL15-IL15Rα Expression Promotes Complete Tumor Regression In A Preclinical Immunocompetent Model Of Lung Cancer (LL2)



LL2 lung tumor cells (1e6) were subcutaneously implanted on both flanks of C57BL mice, and five days post-implantation, animals received a single intravenous dose of 5e6 PFU 3RT 3KO #2, either unarmed or armed with IL15-IL15Rα, or buffer control (n=10 per group). The top graph displays individual tumor volumes and the complete response (CR) rate across groups, while the bottom graph shows tumor expression of IL15-IL15Rα detected via ELISA five days post-treatment with either RT 3KO #2 or RT 3KO #2 encoded IL15-IL15Rα.



Summary

RT-Nova introduces a significant advancement in **true systemic virotherapy** by employing enveloped technology to survive bloodstream and **target all tumors**

RT-Nova is designed with a multimodal Mechanism of action (MOA) which includes: a) direct killing,
b) induction of antitumor immunity,
c) the genetic delivery to tumors of additional immunotherapies (i.e. IL15 superagonist)

Planned **phase 1 clinical trial,** as a monotherapy, targeting metastatic cancers (i.e. Lung cancer) in **Q4 2026**

Aiming to secure Fast-Track, Priority Review, or other **expedited regulatory pathways**

Delivering **off-the-shelf platforms and able to build targeted multiple assets**

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