RedTail, a Systemic Antitumor Virotherapy: Pre-clinical Evaluation of Tumor Targeting, Efficacy, and Safety of Lead Candidate

Building a multimodal immunotherapy able to deliver IL-15(N72D)-IL-15Rα

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Abstract

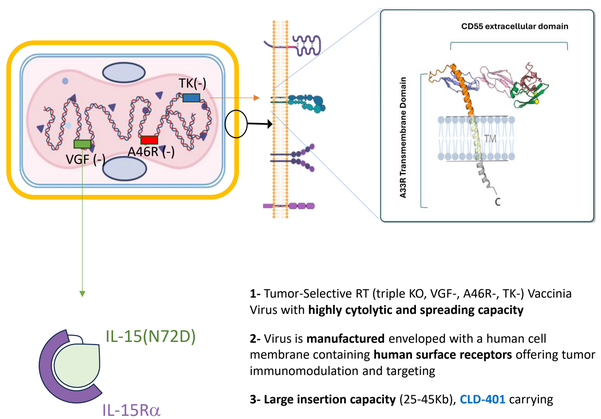
Systemic virotherapy represents a promising frontier in cancer immunotherapy, offering not only direct tumor lysis but also the ability to reshape the tumor immune microenvironment and deliver therapeutic payloads encoded by viruses directly into tumors. A platform enabling systemic delivery would significantly enhance treatment of both primary and metastatic lesions.

To address these limitations, we have selected and developed a novel enveloped vaccinia virus platform based on a complement-resistant strain, termed RedTail vaccinia virus (RT). We further engineered this viral backbone to enable delivery **of a genetic payload to tumor sites. Our lead RedTail compound expresses the IL-15 superagonist in order to** activate **and** enrich immune cell populations within the tumor microenvironment (TME). This strategy aims to maximize therapeutic efficacy while promoting durable anti-tumor immunity and long-term survival benefits.

Stepwise creation of the platform: increasing potency and selectivity, evading immunity, delivering payload

Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
Vaccinia virus engineered for **selective tumor targeting and potent lysis**	Potential for virally-encoded **payload,** delivered in situ (i.e., IL-15 superagonist)	Enveloped virus used for immune protection and tumor targeting: **allows for systemic administration**	Genetic engineering to express **CD55 on envelope** and enhance immune protection	**CLD-401:Systemic administration,** potent and specific tumor lysis, and **IL15 superagonist expression in situ**	New candidates contain **multiple payloads and additional membrane-expressed proteins**

Schematic representation of CLD-401 Lead Candidate Engineering



1- Tumor-Selective RT (triple KO, VGF-, A46R-, TK-) Vaccinia Virus with **highly cytolytic and spreading capacity**

2- Virus is **manufactured** enveloped with a human cell membrane containing **human surface receptors** offering tumor immunomodulation and targeting

3- Large insertion capacity (25-45Kb), **CLD-401** carrying

a) CD55 chimeric anti-complement receptor

b) IL-15(N72D) superagonist,

4- High production of **enveloped viruses with CD55** is critical for enhancing viral survival in the bloodstream, **enabling effective systemic delivery**.

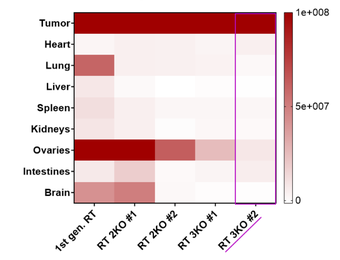
5- IL-15 superagonist enhances the activation and proliferation of natural killer (NK) cells and CD8+ T cells.

6- High therapeutic potential with a multimodal MOA.

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

RT 3KO #2 (TK-, A46R-, VGF-) Preferentially Targets and Amplifies in Tumors and not in Other Major Organs.

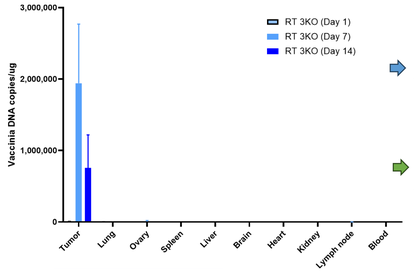
Ex-vivo Signals (Mean Radiance)



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 (**RedTail virus**) preferentially targets and amplifies in tumors and not in other major organs

qPCR analysis of viral DNA in tumor and organs (RT 3KO #2)

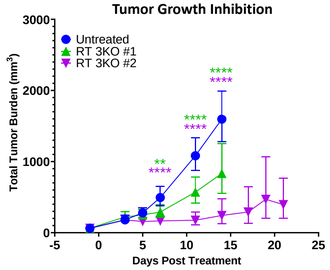


RedTail virus preferentially targets the tumor with 65% of copies found in tumors on day 1 (compared with major organs) in syngeneic mouse lung cancer model.

RedTail virus amplifies preferentially in the tumor.

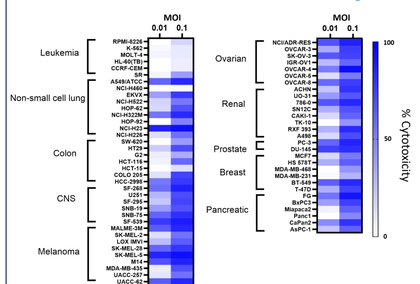
RT 3KO #2 Promotes a More Effective and Lasting Therapeutic Response in Syngeneic Mouse Models of Lung Cancer

Tumor Growth Inhibition



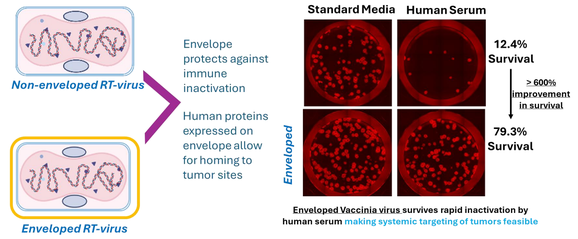
Tumor growth inhibition after administration of different engineered (KO) RT viruses in the syngeneic lung tumor model. Mice were subcutaneously inoculated with LL2 cells, and once tumors reached 60–100 mm³, animals were treated intravenously with a single dose of 5e6 PFU RTs or buffer control (n=5 per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

RedTail Virus Induces a Robust and Direct Killing of Multiple Tumor Cell Types



Analysis of tumor cytotoxicity for RedTail virus across over 60 human tumor cell lines using the NCI-60 panel: Redtail 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.

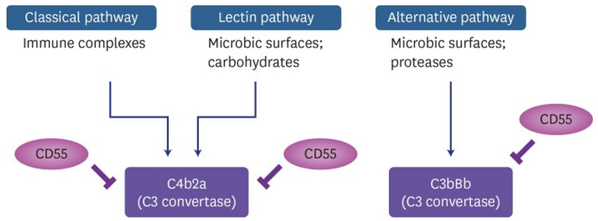
Envelope Maximizes Resistance of RedTail Against Humoral Immunity



Enveloped Vaccinia virus survives rapid inactivation by human serum making systemic targeting of tumors feasible

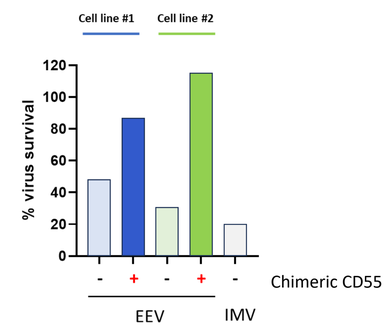
EEV particles but not IMV are resistant to human complement. Standard manufacturing processes focus on producing IMV (intracellular mature virus) rather than EEV (extracellular enveloped vaccinia viruses), necessitating a new process to manufacture EEV. Unlike IMV, EEV particles exhibit resistance to 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.

Role of CD55 in Mediating Resistance to Complement Inactivation



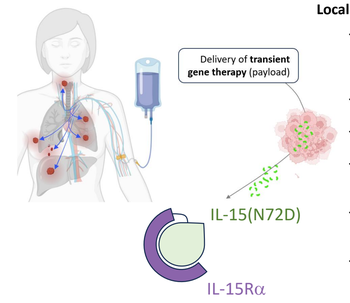
CD55, or Decay-Accelerating Factor (DAF), plays a pivotal role in shielding viruses from complement-mediated neutralization during systemic circulation. By incorporating CD55 into virus envelopes, viruses can effectively inhibit the formation of C3 and C5 convertases, key components of the complement cascade. This inhibition prevents the assembly of the membrane attack complex (MAC), which would otherwise lead to viral lysis.

A33R-CD55 EEV Exhibits High Resistance to Human Complement



Overexpression of chimeric CD55 on the EEV viral membrane enhances complement inactivation, independent of the host cell line. Two distinct host cell lines were seeded at a density of 5 × 10^5 cells per well in 6-well plates. Cells were infected with either RT control (-) or RT expressing Chimeric CD55 (+) virus at a multiplicity of infection (MOI) of 0.5 for 24 hours. EEV-containing supernatants were collected from each infected cell line and subsequently titrated in the presence of 20% human serum, with 20% FBS serving as a control. The RT control cell pellet was harvested, and the released intracellular mature virus (IMV) was included in the experiment

RedTail: A Systemic Virotherapy Approach for Local Delivery of IL-15 Superagonist to Tumor Locations

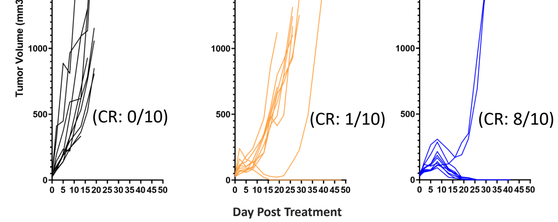


Local production of IL-15(N72D)-IL-15Rα

- ✓ IL-15N72D is a high affinity Interleukin variant with increased biological activity.
- ✓ 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 (IL-15N72D Superagonist)

IL-15(N72D)-IL-15Rα Expression Promotes Complete Tumor Regression In A Preclinical Immunocompetent Model Of Breast Cancer (EMT6)

Tumor Growth Curve



EMT6 breast cancer cells (1e6) were subcutaneously implanted on both flanks of Balb/c mice, and five days post-implantation, animals received a single intravenous dose of 5e6 PFU RT 3KO #2, either unarmed or armed with IL-15-IL-15Rα, or buffer control (n=10 per group). The graph displays individual tumor volumes and the complete response (CR) rate across groups.

Summary

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

The addition of an IL-15 superagonist payload improves the elimination of tumor in syngeneic models

RedTail is designed with a multimodal Mechanism of action (MOA) which includes:

a) tumor targeting,
b) direct killing,
c) induction of antitumor immunity,
d) the genetic delivery to tumors of additional immunotherapies (i.e. IL-15 superagonist)

Planned **phase 1 clinical trial,** as a monotherapy, targeting metastatic cancers (e.g Non small Cell Lung Cancer, Triple Negative Breast Cancer, Ovarian Cancer)

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

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