In Situ Tumor Delivery of IL-15 Superagonist via RedTail Gene Therapy Achieves Durable Tumor Clearance

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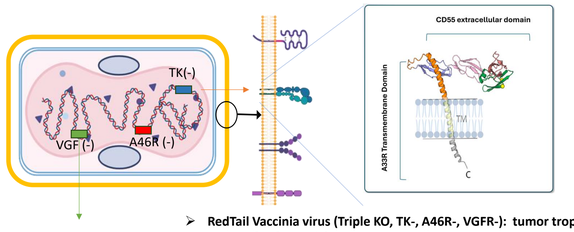
Abstract

RedTail is a next-generation gene therapy platform designed for systemic delivery with enhanced tumor-selective targeting. We previously developed RedTail as an extracellular enveloped form of vaccinia virus, which exhibits significantly greater resistance to immune clearance compared to non-enveloped variants. This immune evasion enables effective systemic administration and robust targeting of metastatic lesions in preclinical models. Our lead candidate, CLD-401, incorporates a novel genetic modification, chimeric CD55, to inhibit complement activation and ultimately improve survival in the bloodstream.

The RedTail platform enables direct delivery of therapeutic genetic payloads into tumors. CLD-401, the first lead candidate of the RedTail platform, transforms tumors into producers of an IL-15 superagonist (IL-15(N72D)-IL-15Rα), a next-generation cytokine known to activate and expand natural killer (NK) cells and CD8+ T cells—both critical for antitumor immunity and with demonstrated

CLD-401: First Lead Candidate from RedTail platform

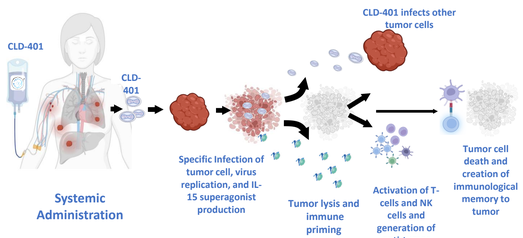


- RedTail Vaccinia virus (Triple KO, TK-, A46R-, VGFR-): tumor tropic and replication restricted to tumor cells
- Selected to produce high levels of extracellular enveloped viruses (EEV)
- EEV naturally constitutes < 1% of the total vaccinia virions, exhibits resistance to complement-mediated neutralization, and is essential for long-range viral dissemination.
- Engineered to express high levels of CD55 to facilitate survival in the complement-rich bloodstream, enabling systemic delivery
- Genetic payload IL-15 (N72D) Superagonist drives potent innate and adaptive immune responses to the tumor

IL-15(N72D)

IL-15Rα

Mechanism of Action



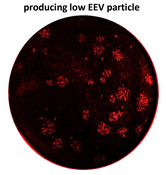
- Systemic administration
- Protected from immune clearance
- Targeted tumor cell lysis and immune priming
- IL-15 superagonist (IL-15 SA) production at the tumor
- Induces innate and adaptive response to the tumors leading to complete responses

CLD-401: Systemic tumor priming with targeted IL-15 superagonist delivery.

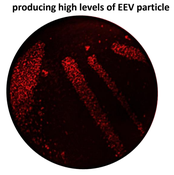
Identifying a Novel Vaccinia Virus Strain with Enhanced EEV Production

Comet assay of two distinct vaccinia viruses

Vaccinia virus strain CAL2 producing low EEV particle



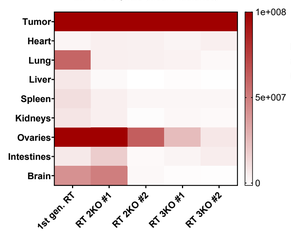
Redtail Vaccinia virus RT producing high levels of EEV particle



Redtail clone

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

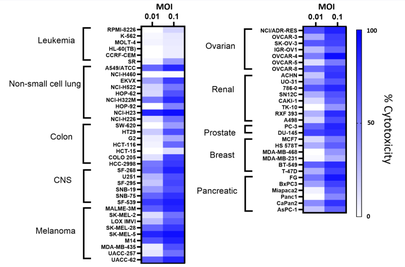
Generation of Tumor selective RedTail virus



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) with KO in TK, A46R and VGF preferentially targets and amplifies in tumors and not in other major organs

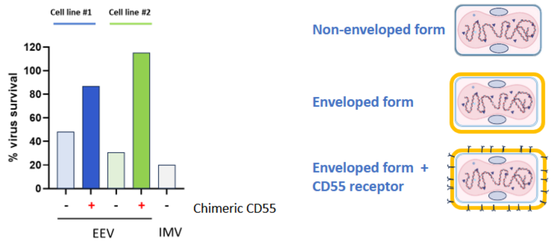
RedTail Induces a Robust and Direct Killing of Multiple Tumor Cell Types



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), with high levels of cytotoxicity in the majority of tumor cell lines.

CD55 membrane expression creates high resistance to immune clearance

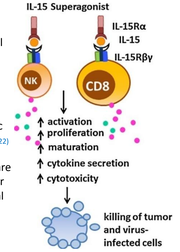
Virus survival in the presence of Human serum



Overexpression of CD55 on EEV membrane protects against complement inactivation and enhances immune evasion. IMV denotes intracellular mature viruses.

IL-15 Superagonist: Proven activator of immunity

- **IL-15 is a powerful growth factor for NK cells (innate) CD8+ T-cells (adaptive)**
  - Unlike IL-2, IL-15 does not expand Tregs or induce activation-induced T-cell death.
- **Anktiva (IL-15 superagonist-Fc) approved for the treatment of BCG-non-responsive non-muscle invasive bladder cancer (NMIBC)**
  - Peak serum concentrations of Anktiva in healthy volunteers and metastatic NSCLC are about **20 pM** (Wrangle J. et al, Lancet Oncol, 2018. Rubienstein MP et al, J Immunol. 2022)
  - Concentrations of Anktiva in the bladder after intravesical administration are expected to be exponentially higher than concentrations in the serum after subcutaneous administration. Intravesical administration results in minimal systemic exposure.
- **CLD-401: IL-15 superagonist chosen as genetic medicine payload**
  - In situ delivery of IL-15 superagonists maximizes therapeutic window.



A major mechanism of resistance to PD-1 therapy is B2M/HLA loss which prevents CD8 T-cell recognition of tumors. IL-15SA activation of NK cells may overcome this resistance mechanism; NK cells can recognize and destroy cells with B2M/HLA loss.

CLD-401 drives tumor-specific IL-15SA expression with minimal systemic exposure

IL-15 SA concentrations (pM) in tumor and organs at multiple time points:

Tumor-bearing model:

Timepoint	Tumor		Liver		Ovary		Lung		Plasma	
	pM	sd	pM	sd	pM	sd	pM	sd	pM	sd
Day 6	62,073.9	16,671.7	13.8	4.6	51.0	85.5	12.7	3.5	15.0	0.8
Day 17	264.6	196.9	3.1	0.4	-	-	-	-	-	-
Day 21	36.4	49.0	2.1	1.0	-	-	-	-	-	-

Non-tumor model:

Timepoint	Liver		Ovary		Lung		Plasma	
	pM	sd	pM	sd	pM	sd	pM	sd
Day 6	2.7	2.2	1.3	1.5	1.2	1.9	3.6	4.3

IL-15 SA concentrations (pM) detected by ELISA after CLD-401 treatment. High tumor concentrations; minimal in liver, ovary, lung, and plasma. Data from EMT6 breast cancer model (Days 6, 17, 21) and non-disease controls (Day 6). Mean ± SD; "–" = not detected.

Tumor-targeted IL-15 SA expression achieves massive tumor enrichment (>60,000 pM) with minimal systemic exposure (~15 pM), overcoming toxicity limits of conventional dosing.

IL-15 SA expression correlates with virus preferential tumor amplification

RedTail virus copy number in tumor and organs at multiple time points:

Tumor-bearing model:

Timepoint	Tumor		Liver		Ovary		Lung	
	copies/ug DNA	sd	copies/ug DNA	sd	copies/ug DNA	sd	copies/ug DNA	sd
Day 6	96,436	12,558	-	-	121	210	-	-
Day 17	10,489	5,741	-	-	-	-	-	-
Day 21	5,085	2,766	-	-	-	-	-	-

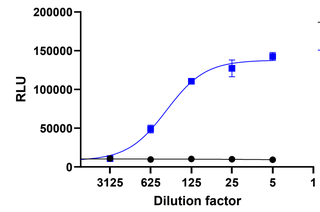
Non-tumor model:

Timepoint	Liver		Ovary		Lung	
	copies/ug DNA	sd	copies/ug DNA	sd	copies/ug DNA	sd
Day 6	-	-	51	79	-	-

Vaccinia virus amplification (copy number) detected by qPCR after CLD-401 treatment showed high tumor enrichment and minimal presence in liver, ovary, lung, and plasma. Data from EMT6 breast cancer model (Days 6, 17, 21) and non-disease controls (Day 6). Mean ± SD; "–" = not detected.

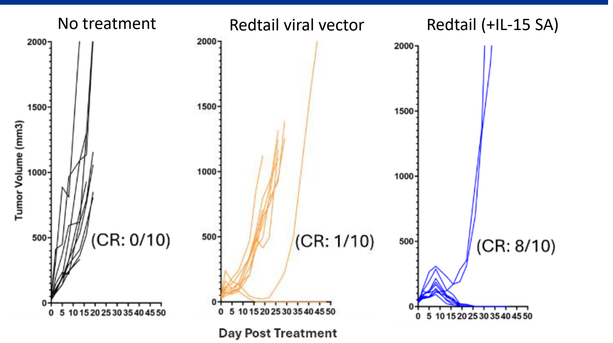
These findings indicate tumor-specific biodistribution and localized IL-15SA expression, supporting systemic administration with minimal off-target exposure.

IL-15 Superagonist produced by CLD-401 shows functional activity



Supernatant from CLD-401-infected A549 cells demonstrated activity in the IL-2/IL-15Rβ bioassay. The response confirms that the supernatant contains functional IL-15 superagonist capable of engaging IL-15 receptor components and triggering downstream signaling in the bioassay cells.

Enhanced Therapeutic activity with In Situ IL-15 Superagonist Expression



Single-dose RedTail with IL-15 superagonist induces tumor regression and complete response in EMT6 breast tumor model.

EMT6 tumor cells (5E6) were subcutaneously implanted on both flanks of Balb/c mice, and five days post-implantation, animals received a single intravenous dose of 5e6 PFU RedTail, either unarmed or armed with IL-15 superagonist, or buffer control (n=10 per group).

Evolving the RedTail Platform

Lead: CLD-401

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- Tumor tropism and replication restricted to tumor cells
- Envelope and CD55 expression facilitate survival in the complement-rich bloodstream, enabling systemic delivery
- IL-15 superagonist payload expression drives potent innate and adaptive immune responses to the tumor

Next-generation

Precision Oncology and Other Diseases

- Envelope engineering for "programmable" targeting
  - Targeting proteins (scFvs, VHHs, etc) expressed in envelope
- Replication in other proliferating cells (ie, activated immune cells)
- Additional payload(s) to impart new biology
  - Immunosuppressive payloads for Inflammatory and immunologic I&I disease