RedTail: A Next- Generation Systemic Platform for Tumor-specific delivery of BiTEs and Immune Activation

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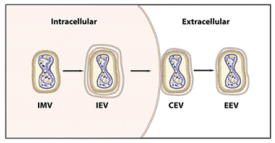
Abstract

RedTail is a next-generation gene therapy platform that combines systemic delivery with high payload expression. The platform uses a tumor specific, replicating extracellular enveloped vaccinia virus (EEV) expressing a chimeric form of CD55, providing resistance to complement and neutralizing antibodies and enabling systemic administration. The viral genome can be engineered to express immune activating payloads such as an IL-15 superagonist (IL-15 SA) and tumor-targeting bispecific T-cell engagers (BiTEs) for localized production within the tumor microenviroment (TME). Tumor selective viral amplification induces cancer cell lysis, T cell infiltration, and delivers high concentrations of immunomodulatory payloads such as IL-15 SA directly into tumors, altering the composition of the TME and potentially overcoming long-standing challenges with T-cell engagement in solid tumors.

Novel Extracellular Enveloped Vaccinia Virus Designed For Systemic Delivery



Adapted from Yu X. et al Viruses 2023
Vaccina Viral Cycle: Intracellular and Extracellular Stages

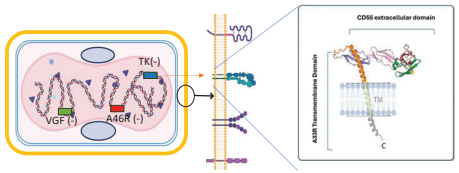
During infection, >99% of virus is Intracellular Mature Virus (IMV), <1% the Extracellular Enveloped Virus (EEV)

RedTail vaccinia virus is selected for:

High production of extracellular enveloped virus (EEV), which resists complement-mediated neutralization and is crucial for long-range viral spread.

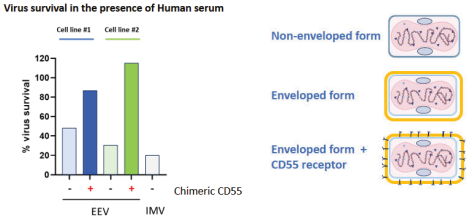
Cell line virus is produced in can affect level of protection.

Generation of Tumor selective RedTail Virus with Triple Knockout and CD55



- Vaccinia virus is tumor tropic, has a rapid and potent lytic cycle, and replicates only in the cytoplasm
- RedTail VV has three knockouts (TK-, A46R-, VGFR-) that restrict replication to tumor cells
- Engineered to express high levels of CD55 to avoid complement and enable systemic delivery
- Large capacity for genetic payload(s)

CD55 membrane expression drives resistance to immune clearance



Complement resistance was restricted to the CD55-overexpressing extracellular enveloped vaccinia virus.

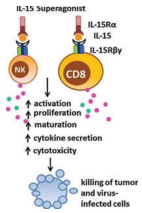
CLD-401: The First Lead Candidate of the Systemic RedTail Platform for Tumor-Localized Expression of an IL-15 Superagonist

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

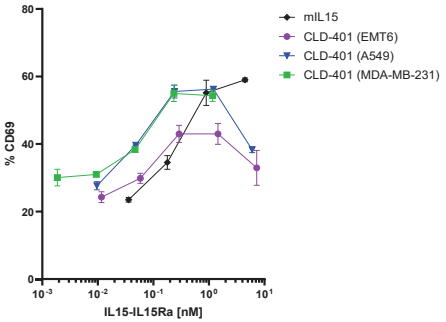
IL-15 Superagonist: Proven Activator of Immunity

- **Anktiva (IL-15 superagonist-Fc) approved for the treatment of BCG-non-responsive non-muscle invasive bladder cancer (NMIBC)**
  - Drug dosed intravesically in bladder cancer at 400 mcg weekly
  - Systemic (Sub Cutaneous) dosing in NSCLC is 80-fold lower than intravesical dose to avoid systemic toxicity
  - Peak serum concentrations of Anktiva in healthy volunteers and metastatic NSCLC are about **20 pM** (Wrangle J. et al, Lancet Oncol, 2018. Rubenstein MP et al, J Immunol. 2022)
- **CLD-401: IL-15 superagonist chosen as genetic medicine payload**
  - In situ delivery of IL-15 superagonists maximizes therapeutic window



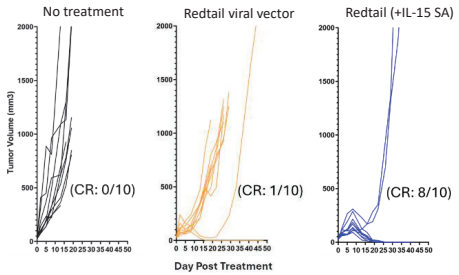
A major mechanism of resistance to PD-1 therapy is B2M/HLA loss which prevents CD8 T-cell recognition of tumors. IL-15 SA activation of NK cells may overcome this resistance mechanism; NK cells can recognize and destroy cells with B2M/HLA loss

Secreted IL-15 Superagonist Activates Murine NK cells



Human IL-15 Superagonist Produced and Secreted by CLD-401–Targeted Tumor Cells Induces Murine NK Cell Activation. Supernatants from CLD-401–infected EMT6 (mouse breast cancer), A549 (human lung cancer), and MDA-MB-231 (human breast cancer) cells were assessed for murine NK cell activation. IL-15 superagonist present in the supernatants induced dose-dependent NK cell activation, as measured by CD69 expression. Recombinant murine IL-15 (mIL-15) served as a positive control.

Single IV dose of RedTail with IL-15 Superagonist Payload Induces Complete responses 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).

Tumor-localized IL-15 SA 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	-	-	-	-	-	-

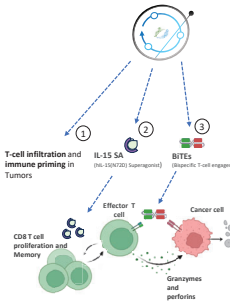
Non-tumor model:

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-15 SA expression, supporting systemic administration with minimal off-target exposure

RedTail Platform Expansion: Tumor-Localized expression of multiple payloads IL-15 SA and BiTEs



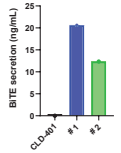
BiTEs generally show poor efficacy in solid tumors because the TME inhibits T-cell engagement and cytotoxicity. Expression of IL-15 SA can remodel the TME and prime T-cells for activity

Therefore, integrating multiple modalities into a single platform helps overcome current challenges

- Systemic delivery
- Tumor targeting
- Oncolysis
- Locally payload production
- Direct activation of T cells

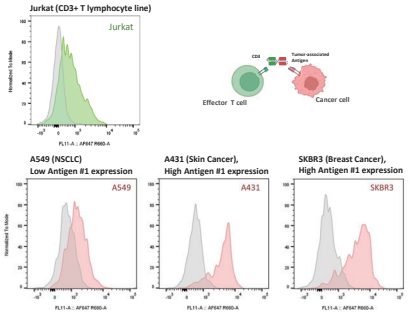
Targeted Cells Infected with Next-Generation RedTail Express and Secrete High Levels of T-Cell Engagers against Solid Tumors

BiTEs secretion by Targeted cells detected by ELISA



CLD-401: IL-15 SA
#1 IL-15 SA + T-Cell Engager (Solid Tumor Target 1)
#2 IL-15 SA + T-Cell Engager (Solid Tumor Target 2)

Binding Activity of Secreted BiTEs Across Multiple Cell Lines



Summary

RedTail Platform Overview

- RedTail is engineered to avoid immune clearance, enabling systemic delivery, while replicating selectively in tumor cells.
- The platform remodels the tumor microenvironment (TME) and delivers therapeutic genes directly to tumors.

Lead Candidate: CLD-401

- CLD-401 is designed to produce high levels of tumor-localized IL-15 superagonist, resulting in durable complete responses.

Pipeline Expansion

- RedTail is designed to remodel the tumor microenvironment and drive high-level, tumor-localized expression of multiple payloads such as an IL-15 superagonist to allow for T-cell activation, along with a tumor-specific BiTE.

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