

Systemic CLD-401 Treatment Drives NK and $\gamma\delta$ T-Cell-Mediated Tumor Eradication via In Situ IL-15 Superagonist Expression

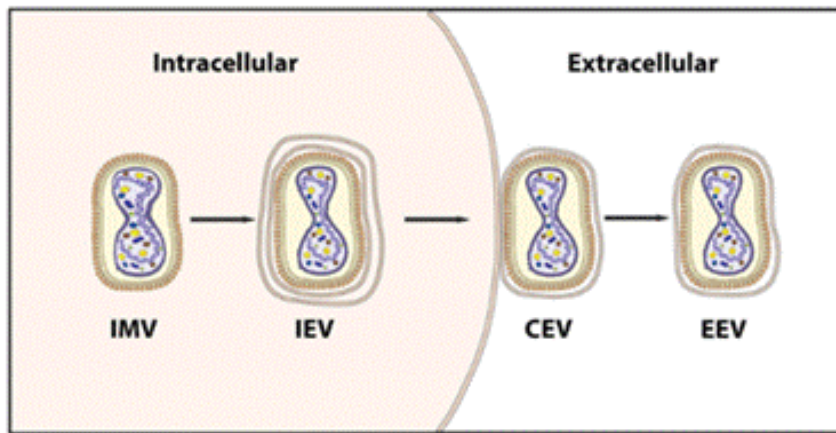


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Abstract

RedTail is a next-generation gene therapy platform engineered for systemic delivery and tumor selectivity. It uses a tumor-specific replication form of extracellular enveloped vaccinia virus (EEV) with chimeric CD55 expression to resist complement and neutralizing antibodies, enabling robust systemic administration. CLD-401, the lead candidate, converts tumors into factories producing IL-15(N72D)-IL-15 α Sushi (IL-15 superagonist, IL-15 SA), a clinically validated cytokine that potently activates NK and CD8⁺ T cells.

Novel Extra cellular enveloped vaccinia virus designed for systemic delivery



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

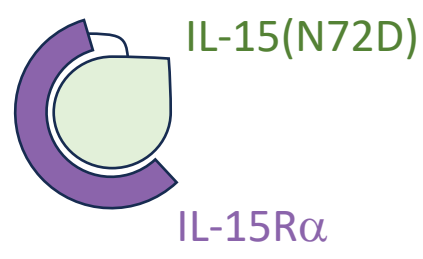
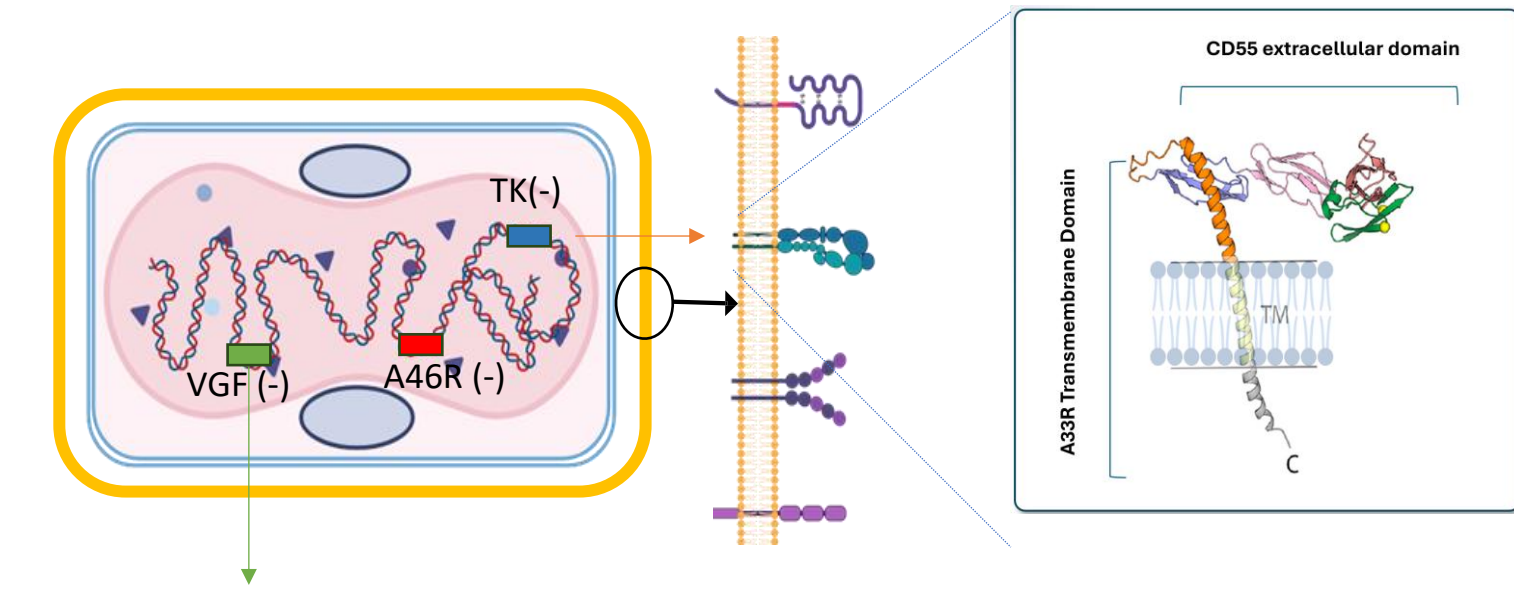
RedTail vaccinia virus is selected for:

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

The host cell line in which the virus is produced can affect its level of protection.

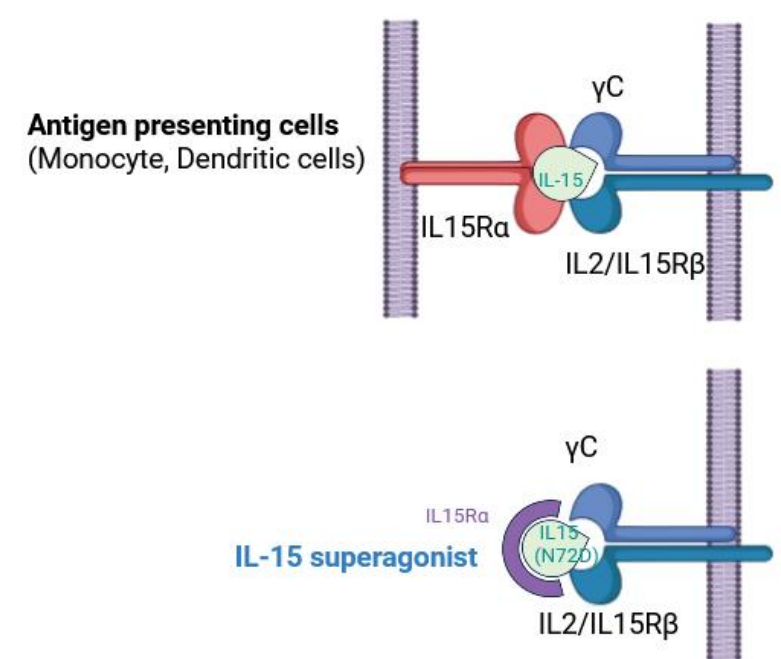
Adapted from Yu X. et al. Viruses 2023
Vaccinia Viral Cycle: Intracellular and Extracellular Stages

CLD-401: First Lead candidate from RedTail platform



- 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-, VGF-) that restrict replication to tumor cells
- Engineered to express high levels of Chimeric A33R \times CD55 to avoid complement and enable systemic delivery
- Large capacity for genetic payload(s)

IL-15 Superagonist (IL-15 SA): Proven activator of immunity



IL-15 is a powerful growth factor for NK, NKT, $\gamma\delta$ T cells (Innate-like) CD8⁺ T cells (Adaptive)

- Unlike IL-2, IL-15 does not expand Tregs or induce activation-induced T-cell death

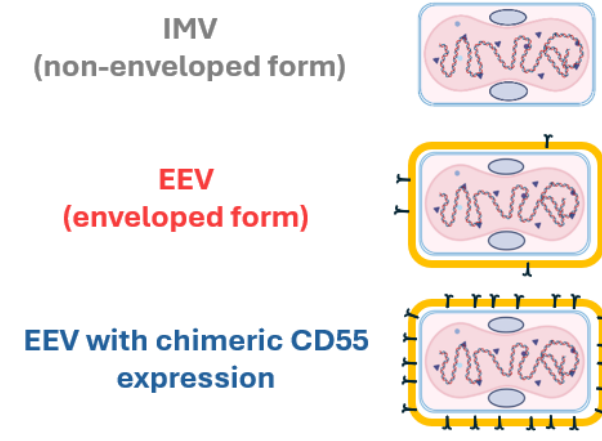
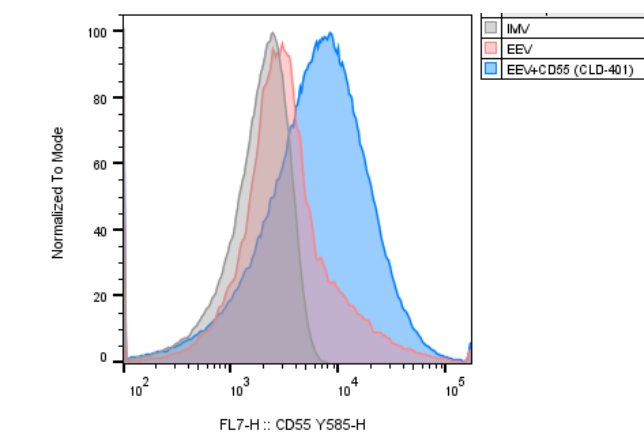
CLD-401: IL-15 SA chosen as genetic medicine payload

- In situ delivery of IL-15 SA maximizes therapeutic window

Chimeric A33R-CD55 expression enables evasion of complement and neutralizing antibodies (nAbs)

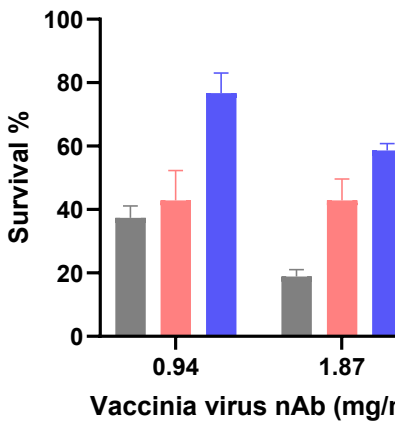
CD55 expression on CLD-401 membrane

Flow virometry analysis shows CD55 expression on EEV membrane of CLD-401

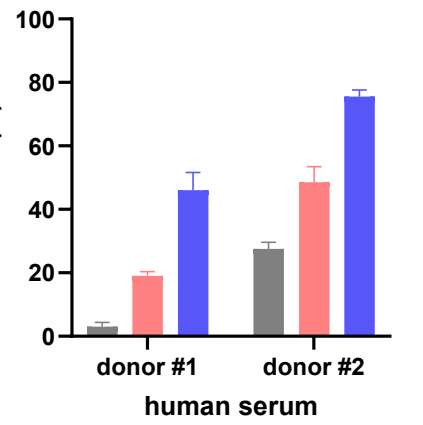


Overexpression of CD55 on CLD-401 EEV membrane protects against complement inactivation and neutralizing antibodies and enhances immune evasion.

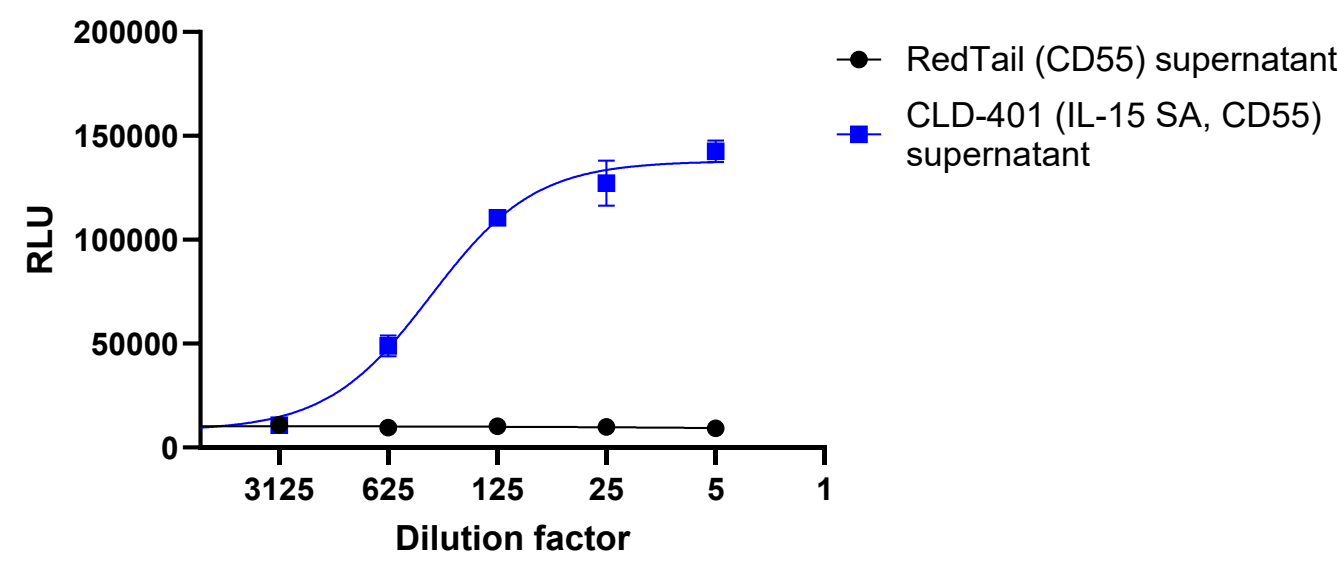
Protection against nAb



Protection against complement

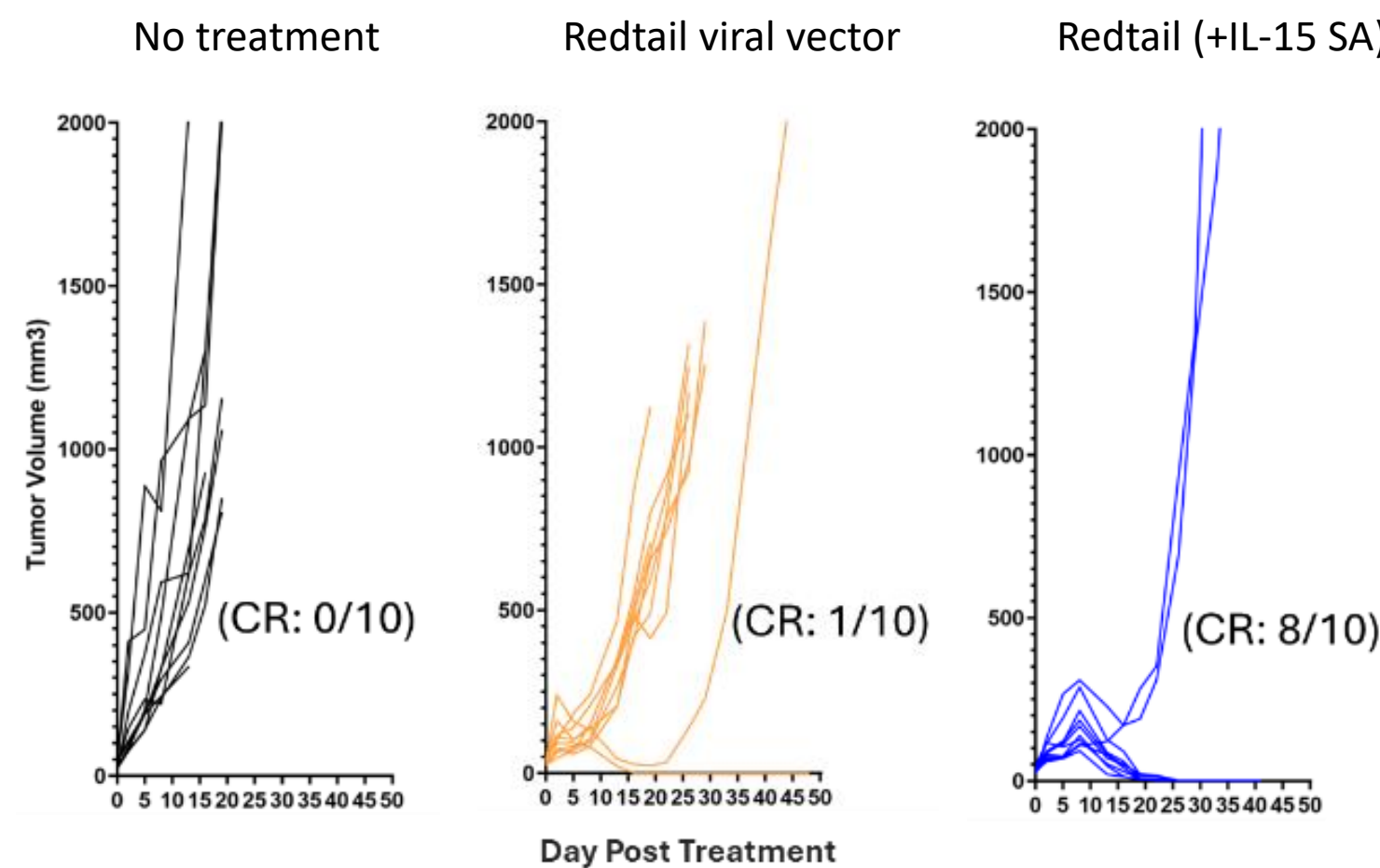


CLD-401 Drives Production of a Functionally Active IL-15 Superagonist



Supernatant from CLD-401-infected A549 cells demonstrated activity in the IL2/IL15R β bioassay cells. The response confirms that the supernatant contains functional IL-15 SA capable of engaging IL-15 receptor components and triggering downstream signaling in the bioassay cells.

Enhanced therapeutic activity with In Situ IL-15 SA expression



Single-dose CLD-401 induces tumor regression and complete response in EMT6 breast tumor model. EMT6 tumor cells (1E6 cells per mouse) 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 IL15 superagonist, or buffer control (n=10 per group).

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

Tumor-localized IL-15 SA production of CLD-401 with minimal systemic exposure

IL-15 SA production (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-disease 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

Tumor-Selective Accumulation of IL-15 SA in CLD-401-treated EMT6 breast tumors confirmed by ELISA. The results show that IL-15 SA was highly concentrated in tumor tissue, while only minimal levels were detected in the liver, ovary, lung, and plasma on Day 6 after treatment. IL-15 SA was completely cleared by Day 17 after treatment. “-” = not detected.

IL-15 SA expression correlates with virus preferential tumor amplification

CLD-401 vaccinia 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-disease model

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

Preferential Tumor Targeting and amplification of CLD-401 in EMT6 breast cancer model. qPCR analysis of CLD-401 vaccinia virus DNA confirmed strong accumulation in tumors with negligible levels in the liver and lung, and trace amounts in the ovary. These findings indicate that CLD-401 achieves tumor-specific IL-15 superagonist production, supporting systemic administration with minimal off-target effects. “-” = not detected.

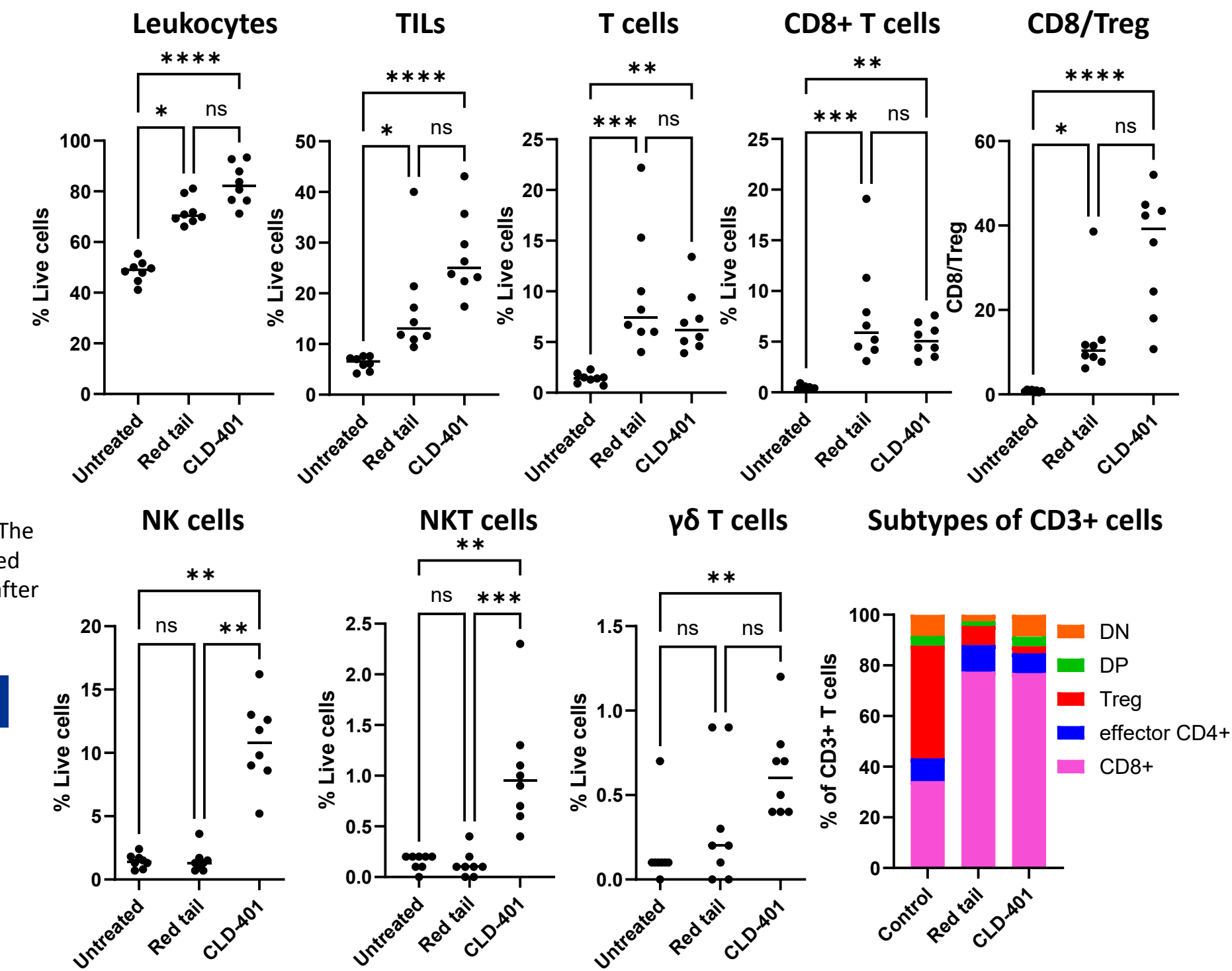
Selective CLD-401 Replication and IL-15 Superagonist Production in Tumor Cells over Human Primary Cells

CLD-401 viral replication and IL-15 SA production (pM) in lung cancer cell line vs. human primary cells:

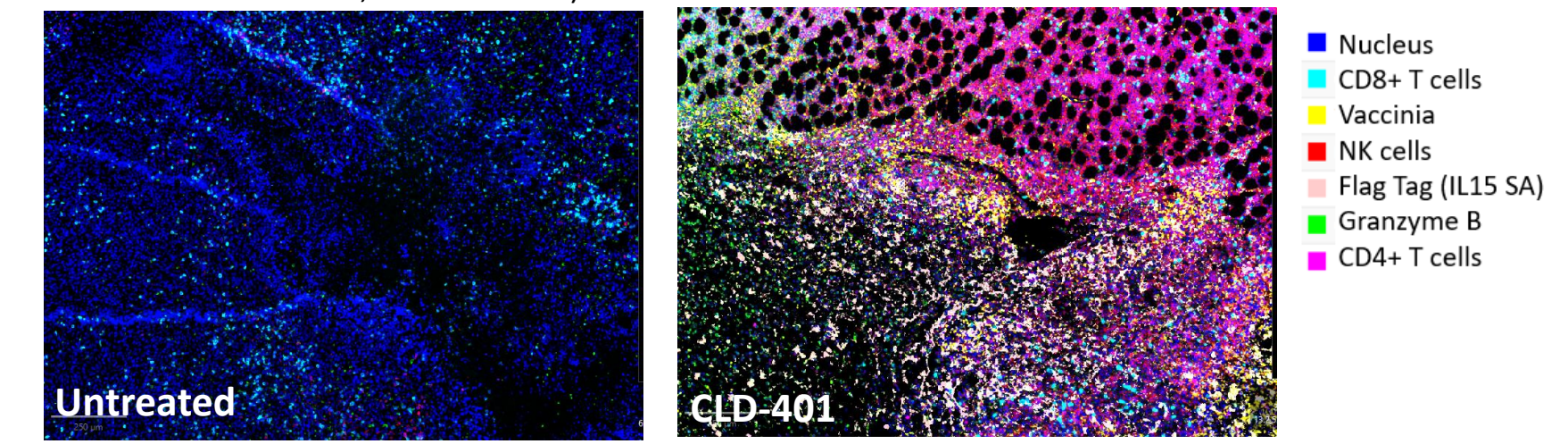
Virus quantity/ Payload production	PFU	A549	Bronchial Epithelial Cells	Dermal Epidermal Keratinocytes	Liver Epithelial Cells	Ovarian Epithelial Cells	Hepatocytes
		Fold change in CLD-401 copy number relative to input	1.4	0.6	1.1	2.2	0.0
IL-15 SA production (pM)	100	1208.2	4.4	3.5	1.2	2.3	2.6
	10	400	-	-	-	-	-
	100	3200	-	-	-	-	-

Selective CLD-401 Replication and IL-15 SA Production in Tumor Cells Supports a Favorable Safety Profile. ELISA analysis showed robust IL-15 SA production in A549 lung cancer cells following CLD-401 infection, while IL-15 SA was undetectable in five tested human primary cell types. Consistent with this, qPCR analysis at 72 hours post-infection (PFU 10 and PFU 100) demonstrated strong viral amplification in A549 cells but no detectable replication in human primary cells. These data indicate tumor-restricted CLD-401 replication and transgene expression, supporting a favorable safety profile with minimal activity in normal human cells. “-” = not detected.

Tumor immune microenvironment change by CLD-401 treatment



CLD-401 enhances NK and innate-like lymphocyte infiltration in EMT6 breast tumor model. EMT6 breast tumor-bearing BALB/c mice were treated systemically with Redtail parent (no Payload) or CLD-401 vaccinia virus (encoding for hIL-15 SA). Tumors were collected 7 days post-treatment and analyzed for immune cell infiltration. Redtail virus increased leukocyte, TIL, CD3⁺ T cell, and neutrophil infiltration and reduced Treg frequency. CLD-401 treatment further suppressed intratumoral Tregs and significantly promoted NK cells and innate-like lymphocytes, including $\gamma\delta$ T cells and NKT cells, reflecting the immunostimulatory effects of the IL-15 superagonist payload. (DN, double negative; DP, double positive, Treg, regulatory T cells; * P<0.05 ** P<0.01 *** P<0.001, **** P<0.0001)



Evolving the RedTail Platform

Lead: CLD-401



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

Next-generation: CLD-501



- Expand payload options – up to three genetic payloads
- Proof-of-concept established with in situ TCE targeting TROP2.
- CLD-501: simultaneous expression of a In Situ anti-TROP2 TCE and IL-15 SA payload(s)