CLD-401: A Systemic Gene Medicine for In Situ IL-15 Superagonist Delivery: Driving NK and γδ T Cell-Mediated Tumor Killing

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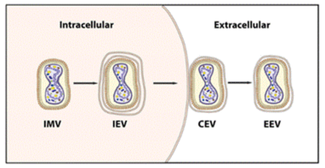
Abstract

RedTail (RT) is a next-generation systemic gene medicine platform leveraging a tumor-selective extracellular enveloped vaccinia virus (EEV) engineered with chimeric CD55 expression to enhance resistance to complement and antibody neutralization. CLD-401, the lead candidate, delivers an IL-15 superagonist (IL-15[N72D]/IL-15Rα sushi domain complex) directly to tumors, driving activation of NK, γδ T, and CD8⁺ T cells within the tumor microenvironment. Tumor-localized IL-15SA expression maximizes antitumor immunity while minimizing systemic exposure and off-tumor toxicity.

Novel Extra cellular enveloped vaccinia virus designed for systemic delivery



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

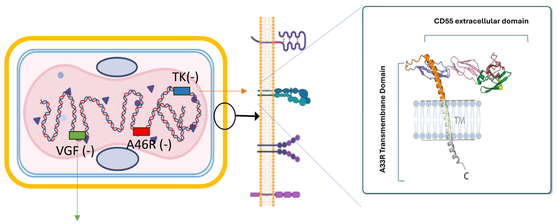
During infection, >99% of virus is Intracellular Mature Virus (IMV), <1% the Extracellular Enveloped Virus (EEV)

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.

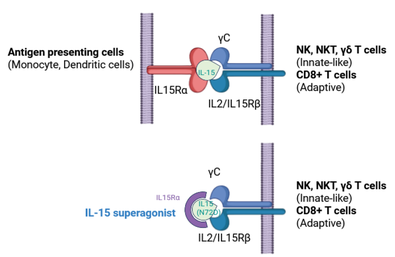
The host cell line in which the virus is produced can affect its level of protection.

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-, VGFR-) that restrict replication to tumor cells**
- **Engineered to express high levels of Chimeric A33RxCD55 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, γδ T cells (innate) 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

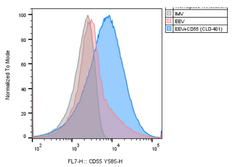
- In situ delivery of IL-15 SA maximizes therapeutic window

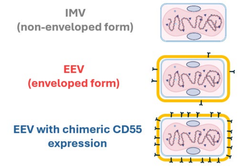
CLD-401: The First RedTail Lead Candidate for Systemic Tumor Priming and In Situ IL-15 Superagonist Delivery

Engineered EEV with chimeric CD55 enables immune evasion

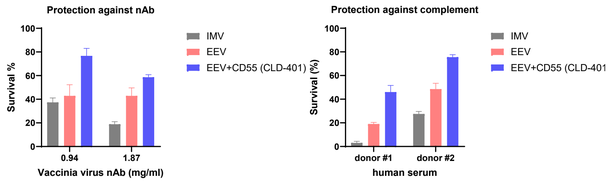
CD55 expression on CLD-401 membrane

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



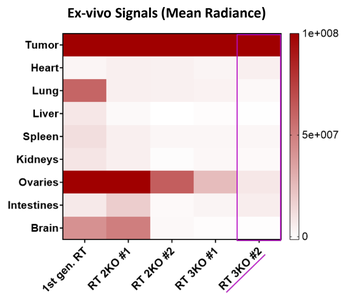


Overexpression of CD55 on CLD-401 EEV membrane protects against complement inactivation and neutralizing antibodies and enhances immune evasion.



CLD-401: Redtail lead candidate for tumor-selective amplification

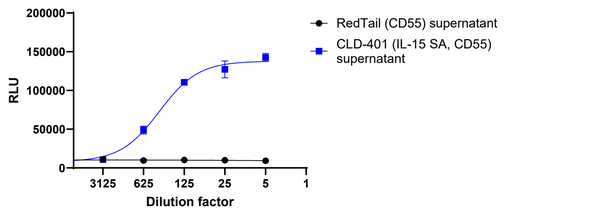
CLD-401: RT 3KO #2 (TK-, A46R-, VGF-) preferentially targeting and amplifying in tumors while reducing off-tumor biodistribution.



Ex vivo fluorescence imaging of tumors and major organs following intravenous administration of engineered RedTail (RT) viruses expressing TurboFP365 in an NSCLC (A549) athymic mouse xenograft model.

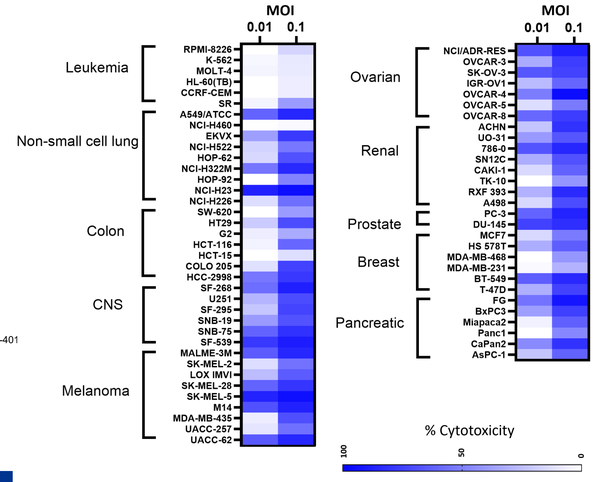
- RT viruses with double (RT-2KO) or triple (RT-3KO) gene knockouts demonstrate reduced off-tumor biodistribution while maintaining tumor-associated signal

CLD-401 drives production of a functionally active IL-15 superagonist



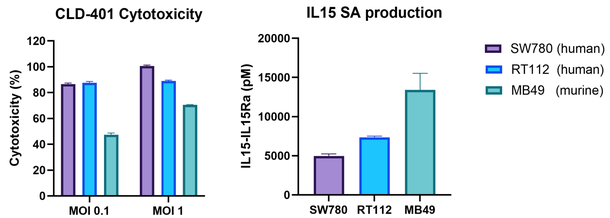
Supernatant from CLD-401-infected A549 cells demonstrated activity in the IL2/IL15Rβ bioassay cells. The response confirms that the supernatant contains functional IL-15 SA capable of engaging IL-15 receptor components and triggering downstream signaling in the bioassay cells.

Broad-spectrum, dose-dependent cytolytic activity in NCI-60 panel



Broad-spectrum cytotoxic activity of RedTail virus across the NCI-60 cancer cell line panel. Human cancer cell lines representing leukemia, non-small cell lung, colon, central nervous system (CNS), melanoma, ovarian, renal, prostate, breast, and pancreatic tumors were infected with RedTail virus at MOIs of 0.01 and 0.1. Cytotoxicity was assessed 72 h post-infection and is presented as a heatmap of percent cytotoxicity. RedTail demonstrated potent, dose-dependent oncolytic activity across a wide range of tumor types, inducing high levels of cell killing in the majority of cancer cell lines even at the lower MOI tested.

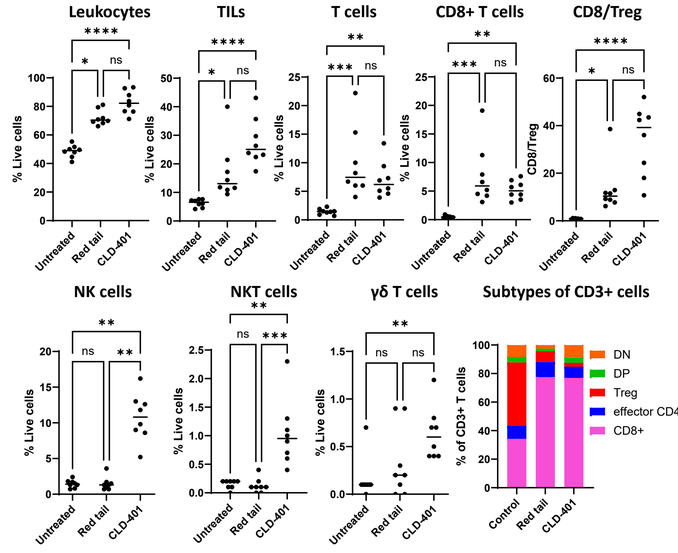
Cytotoxicity and IL-15 SA secretion in human and murine bladder tumor cells



CLD-401-mediated cytotoxicity and IL-15 superagonist (IL-15SA) expression in bladder cancer cell lines. Bladder cancer cell lines (SW780, RT112, and MB49) were seeded in 96-well E-Plates at 10,000 cells/well and infected with CLD-401 at the indicated MOIs. Cytotoxicity was assessed by xCELLigence RTCA at 72 h post-infection (left panel). IL-15SA production was quantified in culture supernatants 48 h post-infection at MOI 0.5 (right panel). Data are presented as mean ± SD.

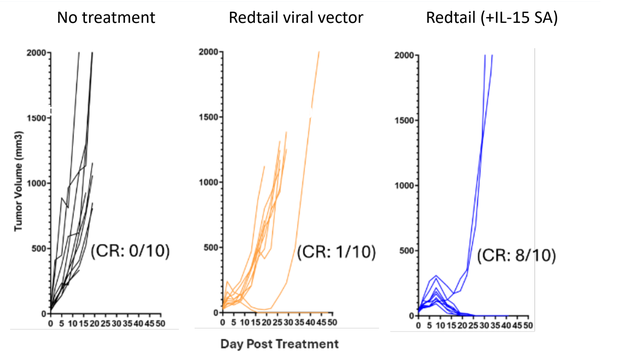
SW780: human low-grade urothelial carcinoma;
RT112: human intermediate-grade urothelial carcinoma;
MB49: murine bladder carcinoma.

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 γδ 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).

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).