CLD-501: An In Situ TROP2 T-Cell Engager and T Cell Amplifier for Solid Tumors

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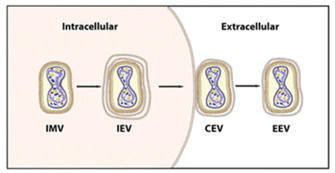
Abstract

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 (TCE) 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 and TCEs, 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

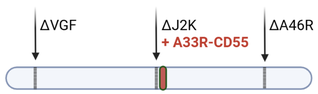
During infection, >99% of virus is Intracellular Mature Virus (IMV), <1% the Extracellular Enveloped Virus (EEV)

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.

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

Generation of Tumor selective RedTail Virus with Triple Knockout and CD55

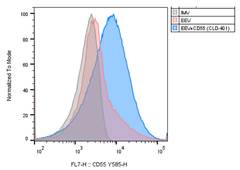


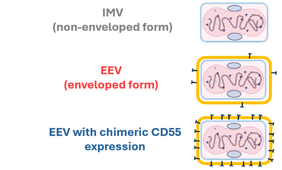
- Vaccinia virus is tumor tropic, has a rapid and potent lytic cycle, and replicates only in the cytoplasm
- RedTail VV has three knockouts (TK-, A46R-, VGF-) that restrict replication to tumor cells
- Engineered to express high levels of chimeric A33RxCD55 in the extracellular envelop of the viral vector to avoid complement and neutralizing antibodies and enable systemic delivery
- Large capacity for genetic payload(s)

Chimeric A33R-CD55 Expression Enables Evasion Of Complement And Neutralizing Antibodies (nAbs)

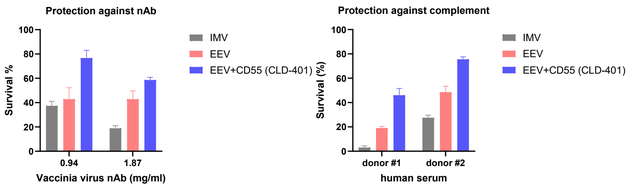
CD55 expression on CLD-401 membrane

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



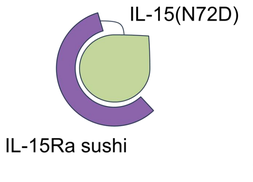


Overexpression of CD55 on CLD-401 EEV membrane protects against complement inactivation and neutralizing antibodies and enhances immune evasion.



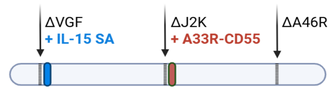
CLD-401: Tumor-Localized Expression of an IL-15 Superagonist

IL-15 Superagonist: Proven Activator of Immunity

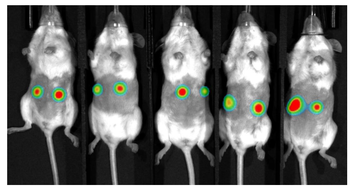


IL-15 is a powerful growth factor for NK cells (innate) CD8+ T-cells (adaptive)

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



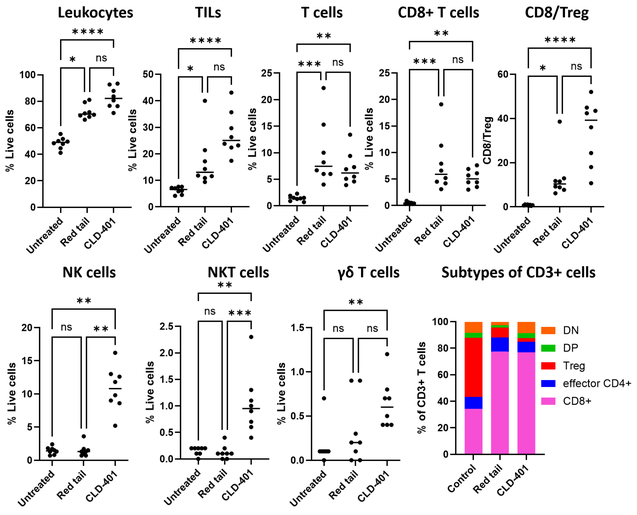
Specific Tumor Targeting and Viral Amplification via I.V. Administration



Preferential Tumor Targeting and amplification of CLD-401 in EMT6 breast cancer model. Single dose of CLD-401 showed selectively accumulates and amplifies in bilateral subcutaneous EMT6 tumors, as visualized by TurboFP635 (top). qPCR of viral DNA and IL-15 SA expression confirmed strong virus amplification and transgene accumulation in tumors with negligible levels in the liver and lung, and trace amounts in the ovary (bottom).

	Tumor		Liver		Ovary		Lung		Plasma	
Timepoint	DNA copies/µg	IL-15 SA (pM)	DNA copies/µg	IL-15 SA (pM)	DNA copies/µg	IL-15 SA (pM)	DNA copies/µg	IL-15 SA (pM)	DNA copies/µg	IL-15 SA (pM)
Day 6	96,436	**62,074**	–	**14**	121	**51**	–	**13**	NA	**15**
Day 17	10,489	**265**	–	**3**	–	–	–	–	NA	–
Day 21	5,085	**36**	–	**2**	–	–	–	–	NA	–

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)

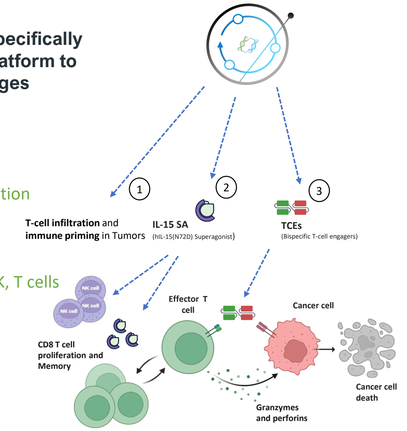
CLD-501: Tumor-localized Expression Of Dual Payloads IL-15 Superagonist and TCEs

T-cell engagers (TCEs) have faced obstacles in the treatment of solid tumors

- ❑ On-target, off-tumor binding drives toxicity
- ❑ Insufficient TCE accumulation in the TME limits efficacy
- ❑ Low quantity and dysfunction of T cells within the TME

In Situ T-cell Engagers were specifically designed using the RedTail platform to overcome these challenges

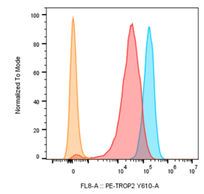
- ➢ Targeting and tumor restricted replication
- ➢ High TCEs expression in the TME
- ➢ High Recruitment and activation of NK, T cells

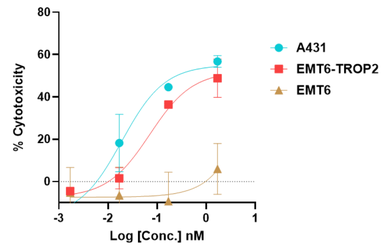


TROP2: A Clinically Validated Tumor Antigen For Targeted Therapies

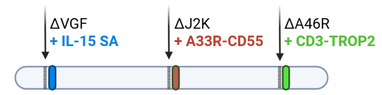
WHAT IS TROP2	WHY IT'S A GOOD TCE TARGET	KEY CHALLENGES
Cell-surface glycoprotein encoded by the TACSTD2 gene	Extracellular domain is directly accessible to bispecific antibodies	On-target/off-tumor toxicity in normal epithelial tissues (skin, GI tract, lung)
Overexpressed in breast (TNBC, HR+), lung, bladder, ovarian, and pancreatic cancers	ADC approvals (sacituzumab) confirm target biology and clinical benefit	Short serum half-life of traditional TCE formats requires continuous IV infusion
Transmembrane protein involved in cell signaling and proliferation pathways	Strong preclinical evidence of T-cell-mediated tumor killing via TROP2 engagement	Immunosuppressive tumor microenvironment (Tregs, MDSCs) may limit T-cell efficacy

Expressed TCE Mediates T-cell Activation And Tumor Cell Killing



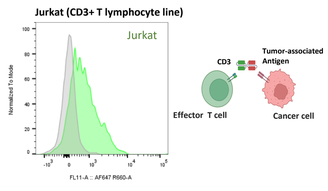


TROP2 expression and TCE-mediated cytotoxicity. Flow cytometry confirms TROP2 expression on A431 and EMT6-TROP2 cells but not parental EMT6 cells (left). TCE induces dose-dependent T-cell–mediated cytotoxicity in TROP2-positive cells, with minimal activity against TROP2-negative EMT6 cells (right).

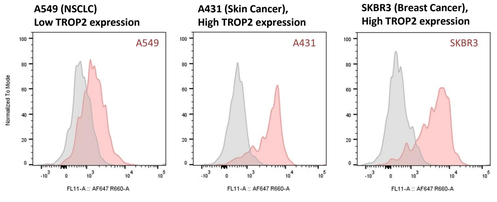


Tumor-Localized TCEs Delivery via Oncolytic Localized TCE Delivery via Oncolytic Vaccinia Virus Enables In Situ Activity and Overcomes Key Challenges of TROP2 TCE

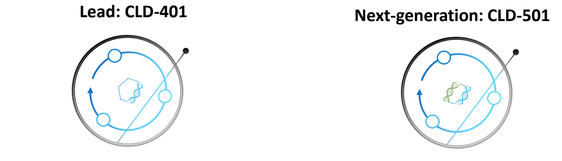
Binding Activity of Secreted TROP2 TCE Across Multiple Cell Lines



Flow cytometry analysis of TCE binding across cell lines with differential TROP2 expression. TCE Binding to CD3 was first confirmed on Jurkat cells ($CD3^+$ T-lymphocyte line) (top). In parallel, binding of the anti-TROP2 TCE was assessed on tumor cell lines with varying surface TROP2 expression levels (bottom).



Evolving the RedTail Platform



Candidate	Genetic Payload	Indications	Discovery	IND Enabling	Phase 1
Systemically Administered (RedTail)					
CLD-401	IL-15 Superagonist	Multiple solid tumors			
CLD-501	• TROP-2 In Situ TCE • IL-15 Superagonist	Multiple TROP2+ solid tumors			
CLD-601	Undisclosed	Myeloma, autoimmune			