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In Situ TCE: Targeting Previously Undruggable Solid Tumor Antigens via Tumor-Restricted T Cell Redirection

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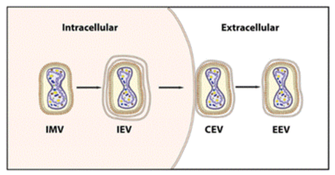
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Abstract

T-cell engagers (TCEs) have transformed hematologic cancer treatment but their use remains limited in solid tumors by off-tumor toxicity, poor tumor exposure, and an immunosuppressive tumor microenvironment (TME). To overcome these barriers, we developed **In Situ TCE, a tumor-selective viral platform that drives local TCE production and TME remodeling.**

- **CLD-401** expresses an **IL-15 superagonist** (IL-15 SA) to promote antitumor immunity and TME remodeling.
- **CLD-501/502** extends CLD-401 by adding a **TROP2- or EpCAM-targeted TCE**, combining:
  - Tumor-selective oncolysis
  - IL-15-mediated immune activation
  - T-cell engagers against TROP2+, EpCAM+ tumor cells

Extracellular Enveloped Vaccinia Virus Designed For Systemic Delivery



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

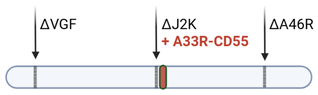
During infection, >99% of virus is Intracellular Mature Virus (IMV), <1% the Extracellular Enveloped Virus (EEV)

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

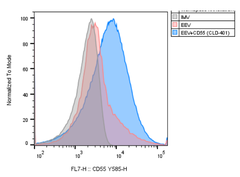


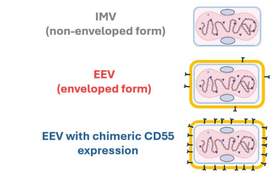
- Vaccinia virus is tumor tropic, has a rapid and potent lytic cycle, and replicates only in the cytoplasm
- RedTail VV has three knockouts (TK-, A46R-, VGF-) that restrict replication to tumor cells
- Engineered to express high levels of chimeric A33RxCD55 in the extracellular envelop of the viral vector to avoid complement and neutralizing antibodies and enable systemic delivery
- Large capacity for genetic payload (25 kbp)

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

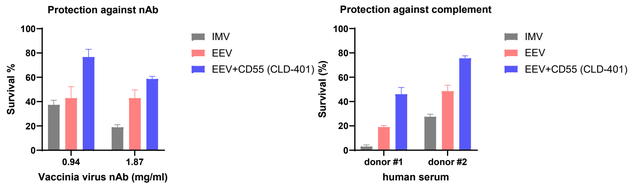
CD55 expression on CLD-401 membrane

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



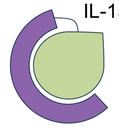


Overexpression of CD55 on CLD-401 EEV membrane protects against complement inactivation and neutralizing antibodies and enhances immune evasion.



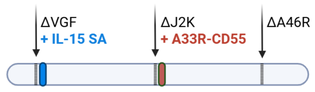
CLD-401: Tumor-Localized Expression of an IL-15 Superagonist

IL-15 Superagonist: Proven Activator of Immunity

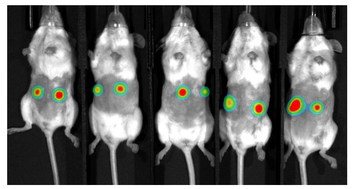


IL-15 is a powerful growth factor for NK cells (innate) CD8+ T-cells (adaptive)

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



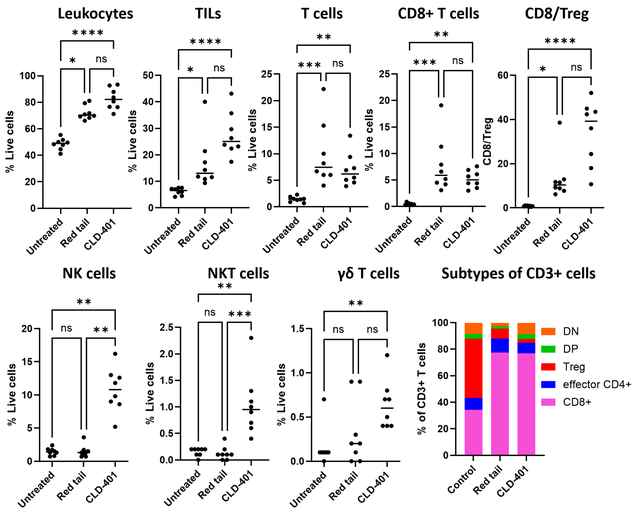
Specific tumor targeting and viral amplification via I.V administration



Preferential Tumor Targeting and amplification of CLD-401 in EMT6 breast cancer model. A single dose selectively accumulates and amplifies in bilateral subcutaneous EMT6 tumors (TurboFP635, top). qPCR confirmed strong viral amplification and IL-15 SA transgene accumulation in tumors, with negligible levels in liver and lung and trace amounts in 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	–

CLD-401 increases tumor T cells, the CD8/Treg ratio and NK cells



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).

Platform: Tumor-localized production of TROP2- or EpCAM-targeted T-cell engagers

Tumor-localized expression of dual payloads: IL-15 superagonist and a T-cell engager

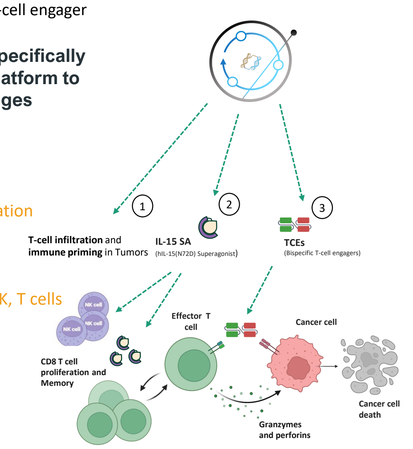
T-cell engagers (TCEs) have faced obstacles in the treatment of solid tumors



- ❑ 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

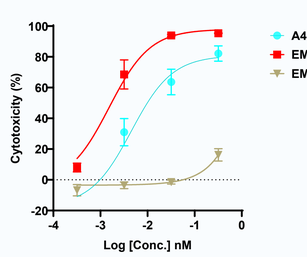
- ➢ Targeting and tumor restricted replication
- ➢ High TCEs expression in the TME
- ➢ High Recruitment and activation of NK, T cells



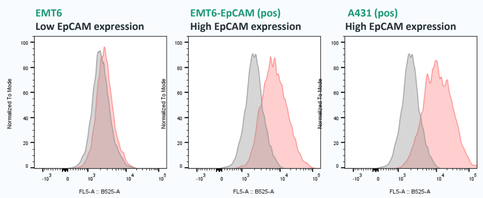


CLD-502: EpCAM TCE + IL15 SA
(Targeting: colorectal, gastric, pancreatic, ovarian)

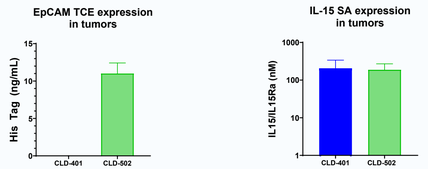
Dose-dependent T cell-mediated cytotoxicity



TCE Binding on EpCAM low and high tumor cell lines



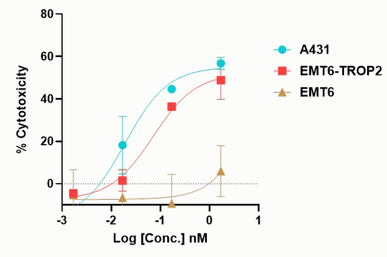
Systemic Delivery Drives Robust Intratumoral Expression of Both IL-15 Superagonist and EpCAM TCE



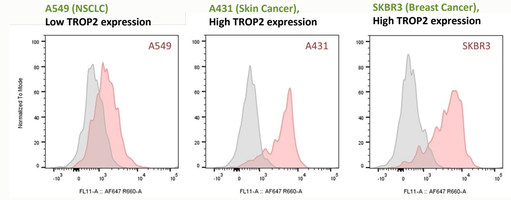
EMT6 tumors were treated i.v. with 4 × 10^6 viral particles. Tumors were harvested 7 days after treatment, and intratumoral expression of EpCAM TCE and IL-15 SA was quantified

CLD-501: TROP2 TCE + IL15 SA
(Targeting: breast, lung, bladder, cervical)

Dose-dependent T cell-mediated cytotoxicity



TCE Binding on TROP2 low and high tumor cell lines



Pipeline

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-502	• EpCAM In Situ TCE • IL-15 Superagonist	Multiple EpCAM+ solid tumors			
CLD-601	Undisclosed	Myeloma, autoimmune			

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