Deciphering Anticancer Mechanisms of Oncolytic Virus-loaded Stem Cells

M. Hammad[1], D. Nguyen[2], A. Seth[1], Y.-C. Yuan[3], H. Ngai[1], I. Minev[2], R. Mooney[1], J. Lara[1], D. Kim[1], L. Flores[1], M. McDonald[1], Y. Kang[2], B. Minev[2], A. Santidrian[2], K. Aboody[1];

[1]City of Hope National Medical Center, Duarte, CA, [2]Calidi Biotherapeutics, San Diego, CA, [3]Department of Computational Quantitative Medicine, City of Hope Comprehensive Cancer Center, Duarte, CA.

Background

Background: Oncolytic viruses (OVs) selectively infect, replicate, and eradicate tumor cells without endangering healthy cells. However, any virus that enters the body will be found and rendered inactive by our immune system. Therefore, cancer-killing viruses don't function properly when given by themselves. Loading therapeutic viruses into tumor-tropic stem cells is a promising solution since they shield the OVs from the immune system until they reach tumor cells and eventually destroy them. Stem cells are potent immunomodulators and, apart from protecting and delivering the cancer-killing viruses, may release additional cell factors that beneficially regulate the tumor microenvironment. In this study, we analyze these factors secreted by clinically relevant stem cells loaded with oncolytic viruses CLD-101 (NeuroNova platform) and CLD-201 (SuperNova platform).

Methods: A neural stem cell (NSC) line was loaded with conditionally replicative adenovirus CRAd-S-pk7 (CLD-101), driven by the *survivin* promotor that is highly expressed in glioma cells. Adipose-derived mesenchymal stem cells (AD-MSC) were loaded with tumor-selective oncolytic vaccinia virus CAL1 (CLD-201). Transcriptomic profiles and cytotoxic effect of stem cell-OVs on cancer cells with and without the human serum were assessed.

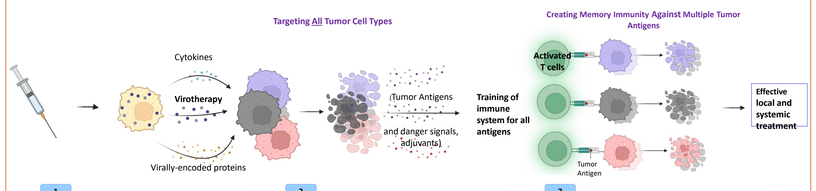
Results: The transcriptomic analysis demonstrated that immunomodulatory cytokines, chemokines, and their receptors are induced after 3 hours of OV infection. OVs kill more than 40 cancer cell lines in-vitro. Stem cells retain cytotoxic effect of OVs on cancer cells after exposure to human serum.

Conclusions: Results suggest that the enhanced therapeutic efficacy of stem cells-OVs vs. naked OVs is at least partially attributable to qualitative and quantitative alterations in the stem cells (including immunostimulatory cytokines and chemokines). These findings advance our knowledge of the molecular mechanisms underlying the immunostimulatory role of OV-loaded stem cells and specifically help to understand the mechanism of action of the promising clinical oncolytic viro-immunotherapies CLD-101 and CLD-201.

Cell-Based Virotherapy for Universal Impact Across Tumor Types

Unprotected viruses are quickly eliminated by the immune system before reaching the tumor cells

Calidi's Novel Cell-based platforms for efficient delivery and potentiation of oncolytic viruses



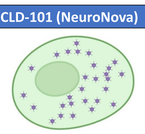
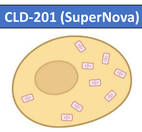
1 **Designed to attack tumor using multimodal combination of MOA.**

1- Immunomodulatory (from cells)
2- Oncolytic (Virotherapy)
3- Additional MOA by virally-encoded proteins

2 **Cell-based virotherapy kills all types of tumor cells (Tumor agnostic).**
1- High therapeutic index of treated lesion/areas (Intratumoral)
2- Low toxicity
3- Strong activation of local and systemic immunity
4- In Situ vaccination

3 **Release of tumor antigens from all tumor types generates a wide and long-lasting anti-tumor immunity.**

Combination with checkpoint inhibitors will ensure durability of the anti-tumor immune response

Therapy	CLD-101 (NeuroNova)	CLD-201 (SuperNova)
		
Delivery vehicle/potentiator	Allogeneic **Neuronal Stem Cells**	Allogeneic **Mesenchymal Stem Cells**
Tumor selective Virotherapy	Adenovirus: CRAd-S-pk7	Vaccinia virus: CAL1
Indication	High Grade Glioma	Advanced Solid Tumors
Product type	Off-the-shelf Localized administration	Off-the-shelf Intratumoral administration

CLD-101 (NeuroNova NSCs)

CLD-101 Protects Adeno Virus Against Human Serum-induced Inactivation

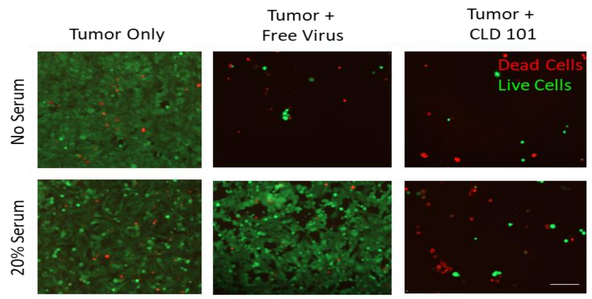


Figure 2. NSCs protect oncolytic virotherapy. Representative fluorescent images of day 7 GL261 brain cancer cell cultures stained with Calcein-AM and Ethidium Bromide to visualize live (green) and dead (red) cells, respectively. Cultures were treated with either free CRAd-S-pk7 or dose-matched NSC-Crad-s-pk7 with and without the addition of 20% human serum. Scale bar = 50µm and applies to all images.

A **CLD-101 Cytolytic Effect**

B **CLD-101 Secreted Cytokines and Chemokines**

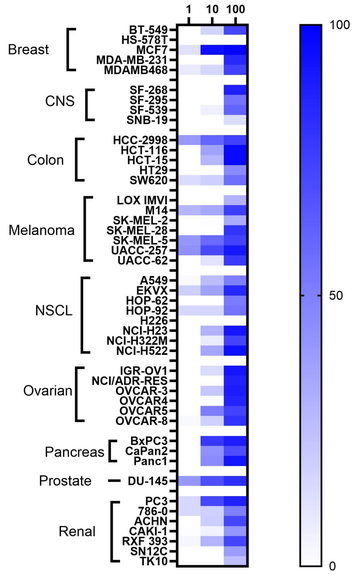


Fig. 3. Cytolysis and RNAseq data for CL101 (A) Cytolysis of 47 human cancer cell lines after being cocultured with CLD-101. Cancer cells were cultured 24 hours before hand and either left untreated (negative control) or treated with CLD-101 with a Multiplicity of Infection (MOI) of 1, 10, or 100. Cytolysis is represented by a decrease of fluorescent calcein accumulation in treated vs control cancer cells, analyzed by the Incucyte S3 system. Cytolytic activity was calculated after 6 days and the percentage is represented as a heat map. (B) RNAseq data for NSC cell-based therapy. The genes of cytokines in NSCs and NSC cell-based therapies was analyzed after being in culture for 24 hours. Data has been Log2 transformed of the average RNA reads.

Highly secreted cytokines and chemokines from CLD-101/CLD-201 and their targets

Symbol	Name	Target Cells	Function
CCL 2	Monocyte Chemotactic Protein 1	M, B, T, NK, DC,	Attracts monocytes and regulates their migration
CXCL 1	Melanoma growth stimulating activity, alpha	N, KC, En, FB	Involved in angiogenesis and inflammation
CXCL 14	Breast and kidney chemokine	M, NK, SC, En	Regulates immune cell migration and executes antimicrobial immunity
CXCL 5	Epithelial cell-derived neutrophil	N	Is a chemokine that attracts neutrophils to sites of inflammation
CXCL 6	Granulocyte chemotactic protein 2	N	Chemokine that attracts neutrophils
CXCL 8	Interleukin 8	N	neutrophil chemotactic factor
IFNE	Interferon type I	NK, B, T	Tumor cells recognition, and T-cell responses.
IL 15	Interleukin 15	NK, T, B	Immune cell activation, proliferation, and survival. Essential for NK cell development
IL 6	Interleukin 6	T, B, other	Involved in inflammation, immune responses, differentiation, and proliferation
IL11	Interleukin 11	HSCs	Promotes hematopoiesis, cell survival, and tissue repair. It is involved in bone marrow function
IL17D	Interleukin 17D	T, NK, N, MC,	Likely contributes to immune responses
IL18BP	Interleukin 18 binding protein	Cells expressing IL-18 receptors	Binds to IL-18 inhibiting its activity. IL-18 is involved in immune regulation and inflammation
IL1B	Interleukin 1 beta	Mp, Ep	Pro-inflammatory cytokine that mediates immune responses, fever, and inflammation
IL32	Interleukin 32	NK, MP, M, T, Ep,	Associated with inflammation and immune responses
IL33	Interleukin 33	Ep, T, B, NK, MP,	Activates immune cells through ST2 receptor

Abbreviation: **N**-neutrophil, **T**-T lymphocyte, **MP**-macrophages, **K**- keratinocytes, **Ep**-epithelial cells, **B**-B lymphocytes, **En**-endothelial cells, **FB**-fibroblast, **DC**-dendritic cells, **M**- monocytes, **HSCs**- hematopoietic stem cells, **MC**-mast cells, **CMs** - Cardiomyocytes, **HEP**-hepatocytes.

CLD-201 (SuperNova AD-MSCs)

CLD-201 Protects Vaccinia Virus Against Human Serum-induced Inactivation

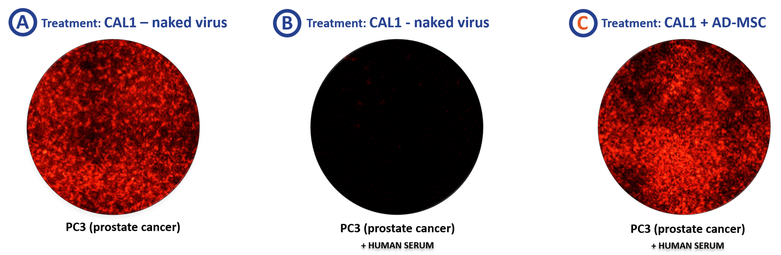


Figure 4. AD-MSCs protect oncolytic virotherapy. Human prostate cancer cells were infected with CAL1-TurboFP or SNV (AD-MSC+CAL1-turboFP) at MOI of 1. (A) Naked CAL1-TurboFP can efficiently kill tumor cells in media without human serum. (B) However, clinical scenario is dramatically different. Human serum/complement can inhibit the oncolytic virus activity by blocking its capacity to infect and kill tumor cells (20% human serum was added). (C) Treatment efficacy was restored when adipose derived stem cells (AD-MSC) are used to protect and potentiate the oncolytic vaccinia virus.

A **CLD-201 Cytolytic Effect**

B **CLD-201 Secreted Cytokines and Chemokines**

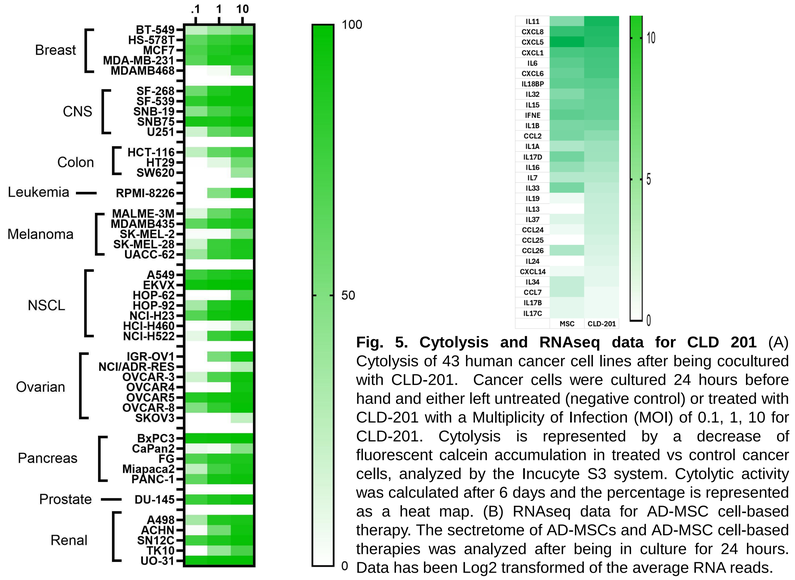


Fig. 5. Cytolysis and RNAseq data for CLD 201 (A) Cytolysis of 43 human cancer cell lines after being cocultured with CLD-201. Cancer cells were cultured 24 hours before hand and either left untreated (negative control) or treated with CLD-201 with a Multiplicity of Infection (MOI) of 0.1, 1, 10 for CLD-201. Cytolysis is represented by a decrease of fluorescent calcein accumulation in treated vs control cancer cells, analyzed by the Incucyte S3 system. Cytolytic activity was calculated after 6 days and the percentage is represented as a heat map. (B) RNAseq data for AD-MSC cell-based therapy. The secretome of AD-MSCs and AD-MSC cell-based therapies was analyzed after being in culture for 24 hours. Data has been Log2 transformed of the average RNA reads.

Future Direction

- Perform proteomics and establish correlations with the acquired transcriptomic data to further evaluate the mechanism of action of CLD-101 and CLD-201.
- Develop murine models aimed at elucidating the in vivo mechanisms underlying the therapeutic efficacy of CLD1101 and CLD-201 against multiple cancer indications and models.

References:

Mooney et al. Enhanced Delivery of Oncolytic Adenovirus by Neural Stem Cells for Treatment of Metastatic Ovarian Cancer. Mol Ther Oncolytics. 2019 Mar 29; 12: 79–92.

Nguyen et al. Development of Allogeneic Stem Cell-Based Platform for Delivery and Potentiation of Oncolytic Virotherapy. Cancers. 2022 Dec 13; 14(24):6136.