Stem Cell Platforms for Enhanced Oncolytic Virotherapy

Antonio F Santidrian[1], Duong Nguyen[1], Yunyi Kang[1], Mohamed Hammad[2], Yate-Ching Yuan[3], Hoi Wa Ngai[2], Rachael Mooney[2], Ivelina Minev[1], Boris R. Minev[1], **Karen Aboody[1]**

[1]Calidi Biotherapeutics, San Diego, CA 92121; [2] Stem Cell Biology and Regenerative Medicine, [3]Department of Computational Quantitative Medicine City of Hope & Beckman Research Institute, Duarte, CA 91010

Abstract

Oncolytic virotherapy has shown promise for multiple cancer types. It involves the use of an oncolytic virus (OV) that infects and selectively kills tumor cells, exposing a myriad of tumor proteins to the immune system. The lysed tumor cells release additional viral particles that continue to infect, amplify within, and kill surrounding tumor cells, ceasing when normal tissue is reached. This self-amplifying and self-limiting treatment is effective against tumor cells that are chemo- or radio-resistant. Most importantly, OV treatments can secondarily stimulate immune system recognition of cancer cells, which can clear residual disease and provide long-term surveillance against relapse. Current OV treatments typically involve repeated administrations of a naked virus. Due to rapid viral clearance by the host immune system, it does not effectively colonize the tumor – resulting in inconsistent, non-durable, and weak anti-tumor immunity. **Calidi's** stem tumor-tropic cell platforms overcome OV limitations by protecting the OVs from immune inactivation, allowing for viral amplification and delivery and distribution of a significantly increased viral load to tumor sites. Stem cells are potent immunomodulators and, apart from protecting and delivering the cancer-killing viruses may release additional cell factors that may regulate tumor microenvironment favoring the effect of the therapy. We analyzed these factors secreted by clinically relevant stem cells loaded with oncolytic viruses CLD-101 (Neuronova platform) and CLD-201 (Supernova platform). CLD-101 was used as adjunct treatment in newly diagnosed glioma patients with promising results (Fares et al, Lancet Oncology 2021). CLD-101 is currently being used in a multi-dose, multi-center trial for recurrent glioma (COH IND 19532; recruiting NCT05139056).

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, are induced after 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: Our findings suggest that the enhanced therapeutic efficacy of stem cells loaded with OV is, at least partially because of qualitative and quantitative alterations in stem cell secretome (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 cell-based oncolytic virotherapies CLD-101 and CLD-201.

Cell-Based Virotherapy for Universal Impact Across Tumor Types

LIMITATIONS of Administering Unprotected Viruses

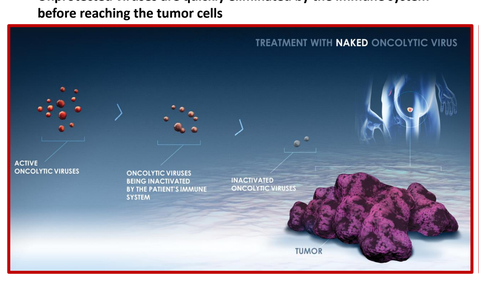
- rapid elimination by host immune system before reaching tumor
- poor viral penetration and distribution through tumor sites
- inability of naked virus to cross normal tissue to reach invasive tumor foci

ADVANCES with Calidi's Novel Cell-based Platforms

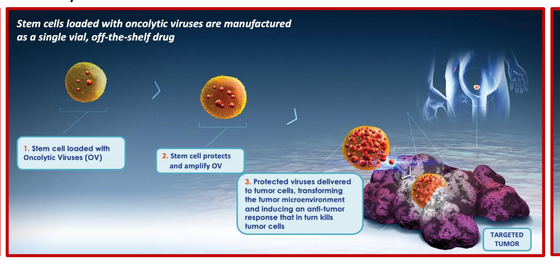
- Protects virus from inactivation
- Allows for amplification of viral particles prior to release
- increase delivery and distribution of virus to tumor sites
- Increased efficacy by oncolysis and stimulation of host anti-tumor immune response

Calidi's Novel Cell-based Platforms

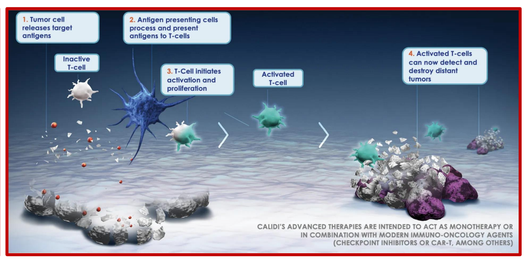
Unprotected viruses are quickly eliminated by the immune system before reaching the tumor cells



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

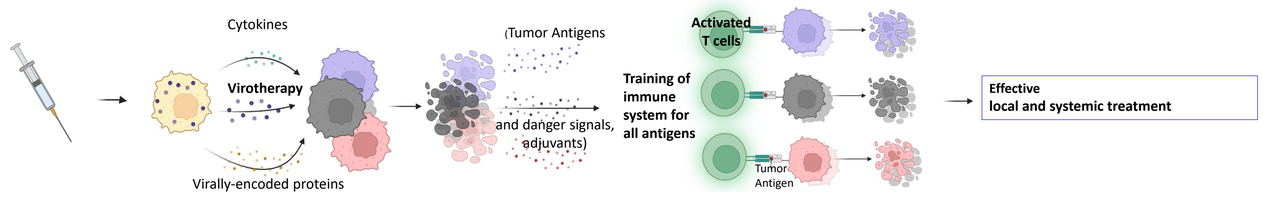


Release of tumor antigens generates a long-lasting anti-tumor immunity



Targeting All Tumor Cell Types

Creating Memory Immunity Against Multiple Tumor Antigens



CALIDI BIOTHERAPEUTICS Cell-based virotherapy clinical pipeline

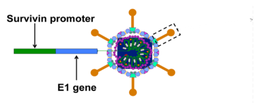
Therapy	CLD-101 (NeuroNova)	CLD-201 (SuperNova)
Delivery vehicle/potentiator	Allogeneic **Neural Stem Cells**	Allogeneic **Mesenchymal Stem Cells**
Tumor selective Virotherapy	Adenovirus: CRAd-S-pk7	Vaccinia virus: CAL1
Indication	High Grade Glioma	Advanced Solid Tumors
Product type	Off-the-shelf Localized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

CLD-101 (NeuroNova)

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

CRAd-S-pk7

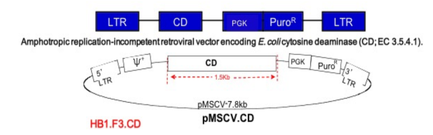
Conditionally Replication-competent Adenovirus (Ad5)
Tumor cell specificity, Survivin promotor

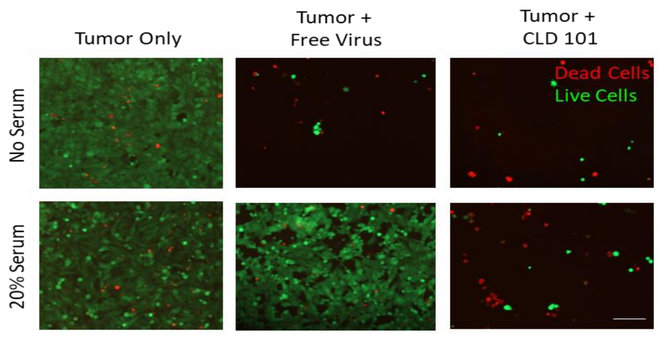


Viral element	Effect
Survivin	Restricts viral replication in tumor cells
E1 gene	Essential gene for viral replication
Pk7 fiber	Enhances infectivity via integrin binding

HB1.F3 CD21 NSC Line

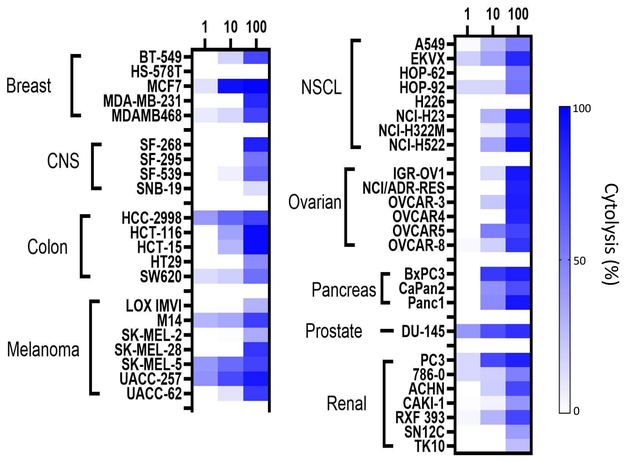
Allogeneic, clonal, expandable, tumor-tropic "off the shelf" product
Genetically and functionally stable, HLA CLII neg
Demonstrated multi-dose clinical safety, tumor-tropism, payload delivery IC in glioma
Preclinical tumor-tropism IV/IP to brain mets. and metastatic solid tumors



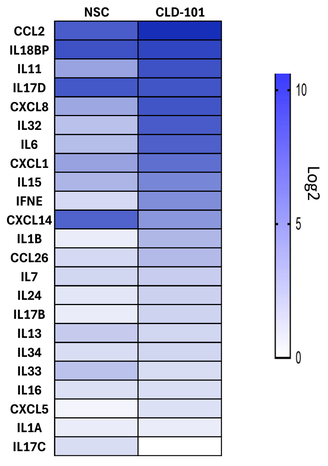


NSCs protect oncolytic adenovirus. Representative fluorescent images of day 7 GL261 brain tumor cell cultures stained with Calcein-AM + Ethidium Bromide to visualize live (green) and dead (red) cells, respectively. Cultures were treated with either free CRAd-S-pk7 or dose-matched NSC-CRAd-S-pk7 +/- f 20% human serum. Scale bar = 50µm

CLD-101 Cytolytic Effect



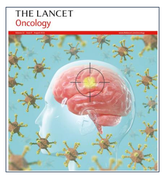
CLD-101 Secreted Cytokines and Chemokines



(A) Cytolysis of 47 human cancer cell lines post-coculture with CLD-101. Cancer cells were cultured for 24 h and then left untreated (neg control) or treated with CLD-101 (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 with percentage represented as a heat map. **(B) RNAseq data for NSC cell-based therapy.** The genes of cytokines in NSCs and NSC cell-based therapies were analyzed post- 24 h in culture. Data has been Log2 transformed of average RNA reads.

CLD-101 CLINICAL TRIALS

Single-dose Phase 1 Trial of CLD-101 in Patients with Newly Diagnosed Glioma



Neural stem cell delivery of an oncolytic adenovirus in newly diagnosed malignant glioma: a first-in-human, phase 1, dose-escalation trial

Jawad Fares, Atiqur U Ahmed, Ilya V Ulasov, Adam M Sonabend, Jason Miska, Catalina Lee-Chang, Irina V Balyasnikova, James P Chandler, Jana Portnow, Matthew C Tate, Priya Kumthekar, Rimas V Lukas, Sean A Grimm, Ann K Adams, Charles D Hébert, Theresa Y Strong, Christina Amidei, Victor A Arrieta, Markella Zannikou, Craig Horbinski, Hui Zhang, Kirsten Bell Burdett, David T Curiel, Sean Sachdev, Karen S Aboody, Roger Stupp, Maciej S Lesniak

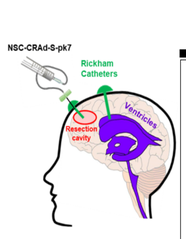
	NSC-CRAd-S-pk7	Stupp et al. (2005)
Phase	1	3
Treatment Regimen	NSC-CRAd-S-pk7 Radiation Temozolomide	Radiation Temozolomide
Number of Patients	12	287
Median PFS	9.1 months	6.9 months
Median OS	18.4 months	14.6 months

Summary of Findings:

- Treatment was well tolerated, no undue toxicity at any dose given, highest dose recommended for phase 2 trials
- Favorable survival outcomes, especially in MGMT unmethylated tumors
- **Evidence of systemic immune responses.** Recruitment of circulating lymphocytes, especially **CD8+ T-cells** in blood and the tumor micro-environment

Multi-dose Phase 1 Trial of CLD-101 in Patients with Recurrent Glioma

PI: Jana Portnow



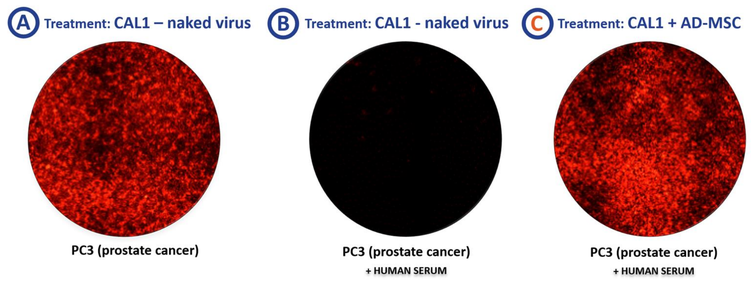
Recruiting NCT05139056

Cycle 1	Cycle 2	Cycle 3	Cycle 4
Day 1 • Collection of samples for correlative studies • Tumor resection • NSC-CRAd-S-pk7 injected into the wall of resection cavity • Placement of 2 Rickham catheters (ICT and ICV) **Day 2** • Collection of samples for correlative studies	**Day 1** • Collection of samples for correlative studies • NSC-CRAd-S-pk7 administered via the ICT Rickham catheter **Day 2** • Collection of samples for correlative studies	**Day 1** • Collection of samples for correlative studies • NSC-CRAd-S-pk7 administered via the ICT Rickham catheter **Day 2** • Collection of samples for correlative studies	**Day 1** • Collection of samples for correlative studies • NSC-CRAd-S-pk7 administered via the ICT Rickham catheter **Day 2** • Collection of samples for correlative studies

Treatment Schema. ICT = intracavitary,; ICV = intraventricular. All participants will be treated with the same dose of CLD-101 (150 x 10^6 NSCs/1.875 x 10^{11} viral particles [VP] per dose) for each weekly cycle.

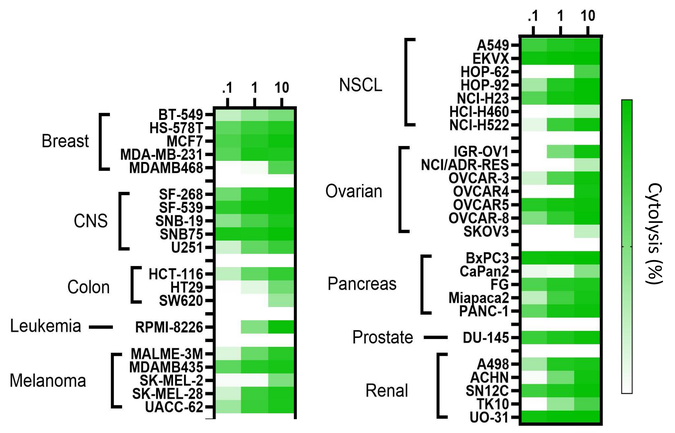
CLD-201 (SuperNova)

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

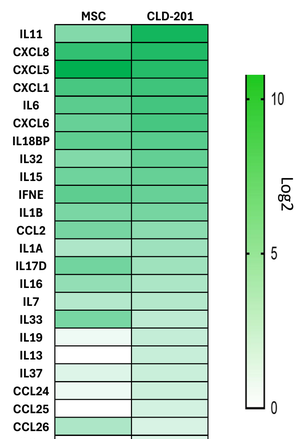


AD-MSCs protect oncolytic virotherapy. Human prostate cancer cells were infected with CAL1-TurboFP or SNV (AD-MSC+CAL1-turboFP) at MOI of 1. (A) Naked CAL1-TurboFP can efficiently kill tumor cells in media without human serum. (B) However, clinical scenario is dramatically different. Human serum/complement 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.

CLD-201 Cytolytic Effect



CLD-201 Secreted Cytokines and Chemokines



(A) Cytolysis of 43 human cancer cell lines after being cocultured with CLD-201. Cancer cells were cultured 24 h and then left untreated (neg control) or treated with CLD-201 with an 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 with percentage 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 h. Data has been Log2 transformed of average RNA reads.

CLD-201: Planned Clinical Development of Allogeneic Platform

A Phase 1/2 study of intra-tumoral administration of CLD-201, in patients with advanced solid tumors

Indications: Skin cancers, Head & Neck, TNBC, soft tissue Sarcoma

PART 1: Dose Escalation in multiple indications

- Classical 3+3 trial design. Three dose levels will be tested,
- Three to 6 patients will be enrolled at each dose level depending on DLTs observed.

PART 2: Expansion in 2-3 Indications

- Ten patients from each of 3 separate indications will be selected from part 1 based on most favorable biological activity
- CLD-201 dose is identified in Part 1 of this trial.

PART 3: Expansion in Best-Responding Indication – Phase 2

- 30 to 50 patients with the best responding indication determined in Part 2
- CLD-201 dose is identified in Part 1 of this trial.

Future Directions

- 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 CLD-101 and CLD-201 against multiple cancer indications and models.

Supernova References:
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Nguyen et al. Development of Allogeneic Stem Cell-Based Platform for Delivery and Potentiation of Oncolytic Virotherapy. *Cancers* 2022 Dec 13; 14(24):6136.
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Portnow et al. Neural stem cell-based anti-cancer gene therapy: a first-in-human study in recurrent high grade glioma patients. *Clin Canc Res* 2017, 23(12), 2951-2960.
Fares et al. Neural stem cell delivery of an oncolytic adenovirus in newly diagnosed malignant glioma: a first-in-human, phase 1, dose-escalation trial. *Lancet Oncol.* 2021 Aug;22(8):1103-1114.
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Portnow et al. Feasibility of intracerebrally administering multiple doses of genetically modified neural stem cells to locally produce chemotherapy in glioma patients *Cancer Gene Ther*. 2021 Apr;28(3-4):294-306.