Non-Clinical Evidence Supporting the Upcoming CLD-201 Clinical Trial: Cell-Based Oncolytic Virotherapy for Multiple Solid Tumors

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Background

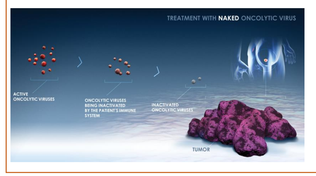
Oncolytic virotherapy is a promising approach that uses viruses to target and destroy cancer cells while activating an anti-tumor immune response. However, a major challenge is the rapid elimination of oncolytic viruses (OVs) by the patient's immune system. Calidi's innovative platform addresses this issue by combining allogeneic stem cells with an OV payload, preventing immune system elimination, and promoting viral amplification at tumor sites. This induces immunogenic cell death and stimulates potent anti-tumor immune responses, effectively targeting primary and metastatic tumors. Prior clinical studies have demonstrated the effectiveness of autologous stem cells loaded with Vaccinia virus CAL1 (ACAM2000) in multiple tumor types, especially when combined with checkpoint inhibitors. However, this approach is costly, lacks scalability and reproducibility. To overcome those limitations, we developed CLD-201 (or SuperNova-1), an innovative concept based on CAL1-loaded allogeneic mesenchymal stem cells, specifically designed for intratumoral administration. This study presents selected non-clinical studies performed to support the upcoming clinical trial to treat multiple solid tumors.

Conclusions

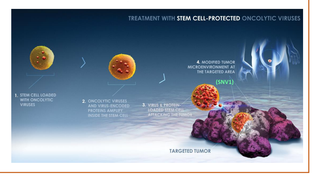
- Animals treated with the maximum tolerated dose (MTD) of 2e6 PFU/animal showed no signs of adverse toxicity and exhibited a reduction in tumor volume compared to the control group.
- No toxicity findings were associated with CLD-201 in the disease-free model. Additionally, virus detection in the lungs was cleared within two weeks following the last CLD-201 treatment.
- A Phase I non-randomized clinical trial is being planned to assess the safety and initial anti-tumor effects of CLD-201, administered intratumorally.

Graphic Abstract

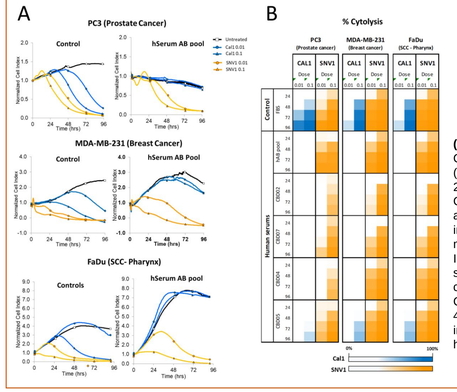
Unprotected viruses are quickly eliminated by the immune system before reaching the tumor cells



Calidi's Novel Supernova-1 for efficient delivery and potentiation of oncolytic viruses



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

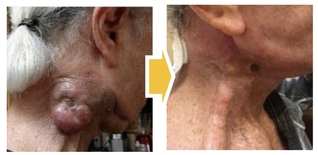


(A) Cytolysis induced by CAL1virus or CLD-201 (SNV1) in cancer cells in 20% human AB pool serum. Cytolysis was automatically analyzed by measuring cell impedance every 15 minutes using the RTCAMP Instrument. **(B)** Heat map shows time courses in which cancer cells were killed by CAL1 and SNV1 at2 4h, 48h, 72h and 96h post infection in presence of 20% human serum

Completed Clinical Safety Study: Autologous settings–Single dose

- **Stem cells (Autologous) loaded with CAL1 treatment was safe and well tolerated.**
- **Tumor regression** of patient with Head Neck tumor (squamous cell carcinoma)

Strong initial signals of efficacy documented (in combination with Checkpoint inhibitors)



Complete trial information: Minev BR et al.
J Transl Med. 2019 Aug 19;17(1):271. doi: 10.1186/s12967-019-2011-3.

Clinical Study In Preparation of Allogeneic CLD-201, off-the shelf-multiple dose

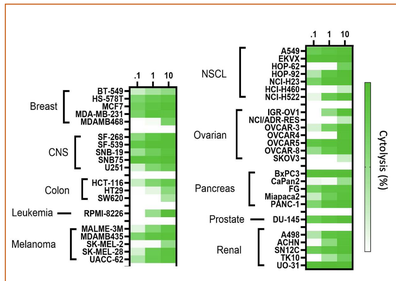
A Phase 1/2 study of intratumoral administration of allogeneic CLD-201 in patients with:
- Head & Neck Squamous Cell Carcinoma (HNSCC)
- Triple Negative Breast Cancer (TNBC)
- Advanced soft tissue Sarcoma

PART 1: Dose Escalation in Five 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 Three 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.

CLD-201 Induces Potent Cytolysis Across Multiple Cancer Types (In Vitro)



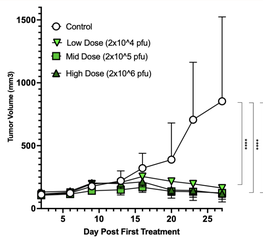
Cytolysis of 43 human cancer cell lines after being cocultured with CLD-201. Cancer cells were cultured 24h and then left untreated (neg control) or treated with CLD-201 with an MOI of 0.1, 1, 10 for CLD-201. Cytolysis is represented by a decrease of fluorescent calcein accumulation in treated vs control cancer cells, analyzed by Incucyte S3 system. Cytolytic activity was calculated after 6 days with percentage represented in a heat map.

CLD-201 Induces Tumor Growth Inhibition in Multiple Cancer Types (In Vivo)

	Cell Line	Cancer Type	Growth Inhibition (%)	Model
Human	PC3	Prostate	59	Xenograft
	MDA-MB-231	Breast	98	Xenograft
	FaDu	Head & Neck SCC	68	Xenograft
	HT-1080	Sarcoma	95	Xenograft
Mouse	EMT-6	Breast	69	Syngeneic
	B16-F10	Melanoma	78	Syngeneic
	CT-26	Colon	77	Syngeneic
	Tramp-C2	Prostate	99	Syngeneic
	RM-1	Prostate	57	Syngeneic

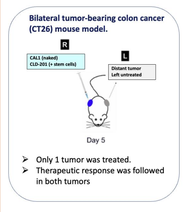
All indicated tumor cell lines were subcutaneously implanted in mice (either with immunocompetent or immunocompromised immune system) to form tumor. When the tumor volume reached around 60-150 mm^3, mice were treated intratumorally with 1e6 cells of CLD-201 (n=7-10). Tumor volume was measured 3 times per week. Mice were sacrificed if the tumors were bigger than 2000 mm^3.

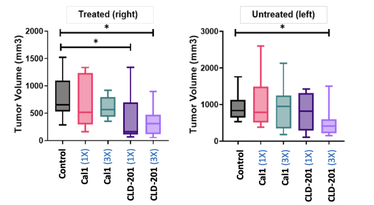
Therapeutic Efficacy of CLD-201 at different dose concentrations



Inhibition of Tumor Growth by CLD-201 treatment in Triple Negative Breast Cancer (MDA-MB-231) Xenograft Model. Athymic nude mice were subcutaneously inoculated with MDA-MB-231 cells in the right flank. Animals with tumor between 50-150 mm^3 were intratumorally treated on Day 1, Day 8 and Day 15 with CLD-201 (SNV1) at varying concentrations (2e4 PFU, 2e5 PFU, 2e6 PFU) or subcutaneous injection with buffer control (n=10 per group). The findings demonstrate a dose-dependent effect, and significant inhibition of tumor growth at every dose level.

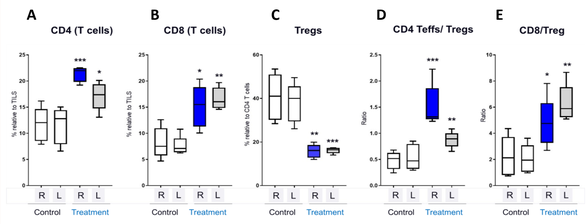
Three (3x) Intratumoral Administrations of CLD-201 Induces Strong and Durable Systemic Antitumor Immunity





SNV1 inhibits treated and distant untreated tumors. Female BALB/c mice were inoculated murine colon cancer cells (CT26) in the right and left abdominal flank regions. Once tumors reached the volume about 50 mm3, animals were treated with 1e6 SNV1 cells (10 PFU/cells) or with 1x107 PFU of CAL1 vaccinia virus one or three times, every 2 days. Only right tumor was treated. Box plot data shows both right and left tumor size 12 days after treatment. Statistical significance was analyzed between treated groups compared to control group using nonparametric Mann-Whitney test, *P < 0.05

Local Administration of CLD-201 Induces Both Robust Local And Systemic Immune Cell Infiltration



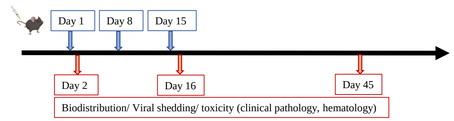
Tumor infiltrating lymphocyte (TIL) analysis in treated and untreated tumors. Five days after the treatment, tumors from 5 controlled and 5 treated animals were excised and enzymatically digested to isolate TILs. The flow cytometry analysis demonstrated statistically significant treatment-related proportional increases in the fractions of total infiltrating CD4 (**A**) and CD8 (**B**) T cells and decreases in the CD4+CD25+Foxp3+ Tregs (**C**). The changes in the T cell compartment were further associated with improved ratios of CD25+Foxp3- CD4+ Teff to Tregs (**D**) and CD8 T cells to Tregs (**E**). These changes in the TME are consistent with conditions favoring direct and indirect oncolysis through potentiation of adaptive anti-tumor immunity. Moreover, similar favorable changes in the fractions or ratios of immune infiltrates can be observed in both the treated (right) and distant untreated tumors (left), providing mechanistic basis for the observed potent abscopal effects.

Non-clinical studies: Disease free model - CLD-201 intravenous administered

ANIMAL MODEL: Disease free immunocompetent C57Bl/6J mice

CLD-201 (SNV1) QW for 3 doses, **Intravenous** administration

Groups: Control
Low Dose (2e4 pfu)
Mid Dose (2e5 pfu)
High Dose (2e6 pfu)



Biodistribution and Shedding (CAL1 Virus)

- After intravenous administration CAL1 virus detection in the lungs was cleared within two weeks after systemic administration.

Day	Dose PFU/mice	Subject ID	Mean Copies/µg DNA: Lungs	Liver	Kidneys	Heart	Brain	Spleen	Eyes	Thymus	Lymph Node	Blood	Tumor/ Ovaries	Salivary Gland	Faeces	Pooled Urine
2	2e6 (High)	396	627	-	-	-	-	-	-	-	-	-	-	-	-	-
		397	3875	24	-	-	-	-	-	-	-	-	-	-	-	-
		398	211	-	-	-	-	-	-	-	47	-	-	-	-	-
		399	-	-	-	-	-	-	-	-	51	-	-	-	-	-
		400	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		426	3047	19	-	-	-	-	-	-	-	-	-	-	-	-
		427	5000	61	-	-	-	-	-	-	-	-	-	-	-	-
		428	6881	57	-	44	-	-	-	-	-	40	-	22	-	-
		429	5346	53	-	-	-	-	-	-	66	-	-	-	-	-
		430	2857	39	-	-	-	-	-	-	381	45	-	-	-	-
16	2e6 (High)	401	280	-	-	-	-	-	-	-	-	-	-	-	-	-
		402	219	-	-	-	-	-	-	-	-	-	-	-	-	-
		403	89	-	-	-	-	16	-	-	-	-	-	-	-	-
		404	26	-	-	-	-	13	-	-	-	-	-	-	-	-
		405	30	-	-	-	-	-	-	-	-	-	-	-	-	-
		431	71	-	-	-	-	18	-	-	-	-	-	-	-	-
		432	366	-	-	-	-	19	-	-	-	-	-	-	-	-
		433	333	-	-	-	-	27	-	-	-	-	-	-	-	-
		434	365	-	-	-	-	-	-	-	-	-	-	-	-	-
		435	92	-	-	-	-	-	-	-	-	-	-	-	-	-
45	2e6 (High)	406	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		407	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		408	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		409	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		410	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		436	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		437	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		438	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		439	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		440	-	-	-	-	-	-	-	-	-	-	-	-	-	-

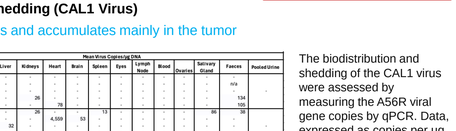
The biodistribution and shedding of the CAL1 virus were assessed by measuring the A56R viral gene copies by qPCR. Data, expressed as copies per µg of DNA. Samples were collected from blood, urine, faeces and tissue samples on Days 2, 16, and 45 after first CLD-201 administration.
After systemic administration, data showed the virus was present mainly in the lungs on Day 2, with clearance after the final dose leading to complete elimination after a month. In Day 16 (1 day after last dose), viral DNA was additionally found in Spleen indicating clearance of the virus. No virus DNA was found in Day 45 indicating complete clearance of the virus in immunocompetent subjects.

Non-clinical studies: Human tumor bearing study –intratumoral administration

ANIMAL MODEL: Human Carcinoma Bearing Athymic Nude -Foxn1^{nu} Mice

CLD-201 (SNV1) QW for 3 doses, **Intratumoral** administration

Groups: Control
Low Dose (2e4 pfu)
Mid Dose (2e5 pfu)
High Dose (2e6 pfu)



Biodistribution and Shedding (CAL1 Virus)

- CAL1 virus amplifies and accumulates mainly in the tumor

Day	Dose PFU/mice	Subject ID	Mean Virus Copies/µg DNA: Tumour	Lungs	Liver	Kidneys	Heart	Brain	Spleen	Eyes	Lymph Node	Blood	Salivary Gland	Faeces	Pooled Urine
Day16	2e4 (low)	5c004-01f	11,988,889	-	-	-	-	-	-	-	-	-	-	-	-
		5c004-02f	6,956,893	-	-	-	-	-	-	-	-	-	-	n/a	-
		5c004-03f	5,275,110	33	-	-	-	-	-	-	-	-	-	-	-
		5c004-04f	5,471,666	-	-	26	-	-	-	-	-	-	-	134	-
		5c004-05f	-	-	-	-	78	-	-	-	-	-	-	105	-
	2e5 (medium)	5c003-01f	1,900,813	-	-	-	26	-	-	13	-	-	-	86	38
		5c003-02f	4,744,975	-	-	-	-	4,559	53	-	-	-	-	-	-
		5c003-03f	1,499,503	-	32	-	-	-	-	-	-	-	-	-	-
		5c003-04f	2,479,599	-	-	-	-	-	-	-	-	-	-	-	-
		5c003-05f	1,996,505	-	-	-	-	-	79	-	59	-	-	-	-
	2e6 (high)	5c002-01f	2,962,550	-	-	-	-	-	-	-	-	-	-	-	-
		5c002-02f	12,399,687	-	96	-	-	-	-	-	47	-	-	-	-
		5c002-03f	181,480	-	32	-	-	-	-	-	-	-	-	-	-
		5c002-04f	2,113,721	-	28	-	-	-	-	-	-	-	-	-	-
		5c002-05f	7,529,760	-	-	-	-	-	-	-	-	-	-	-	-
Day22	2e4 (low)	5c004-06f	5,997,743	-	-	-	-	-	-	-	-	-	-	-	-
		5c004-07f	5,101,569	-	-	-	-	-	-	-	-	-	-	-	-
		5c004-08f	8,677,222	-	-	-	-	-	-	-	-	-	-	-	-
		5c004-09f	3,414,564	-	-	-	-	-	-	-	-	-	-	-	-
		5c004-10f	7,338,881	60	23	-	25	-	-	-	-	-	-	-	-
	2e5 (medium)	5c003-06f	1,100,113	-	-	-	-	-	-	-	-	-	-	-	-
		5c003-07f	2,244,194	18	-	-	-	41	43	-	-	-	-	-	-
		5c003-08f	12,583,125	21	-	-	-	-	-	-	-	-	-	-	-
		5c003-09f	8,314,732	126	30	-	-	1,150	55	-	-	-	-	87	281
		5c003-10f	4,053,736	-	-	-	-	-	-	-	41	-	-	-	-
	2e6 (high)	5c002-06f	2,943,650	-	-	-	-	-	-	-	-	-	-	n/a	-
		5c002-07f	10,894,360	-	-	-	-	-	-	-	-	-	-	438	-
		5c002-08f	3,212,441	-	-	-	-	-	-	-	-	-	-	-	-
		5c002-09f	1,499	48	-	-	-	-	-	-	-	-	-	-	-
		5c002-10f	8,947,500	-	-	-	-	-	-	-	-	-	-	578	-
Day29	2e4 (low)	5c004-11f	10,457,805	-	-	-	-	264	36	-	-	-	58	-	-
		5c004-12f	1,154,079	-	-	-	-	-	-	-	-	-	29	-	-
		5c004-13f	8,611,221	-	-	-	-	-	-	n/a	-	78	57	27,261	-
		5c004-14f	5,939,137	-	45	-	-	-	-	-	-	-	-	-	-
		5c004-15f	30,838,961	129	-	-	-	-	-	-	-	123	-	-	13,494
	2e5 (medium)	5c003-11f	7,506,733	48	-	-	-	267	-	n/a	328	38	55	509	-
		5c003-12f	14,883,822	-	-	64	-	-	-	-	-	-	-	-	4,097
		5c003-13f	5,988,659	-	-	-	-	-	-	-	-	-	-	-	-
		5c003-14f	5,285,473	35	461	11,188	43	-	-	39	-	225	33	53	-
		5c003-15f	7,881,267	-	143	-	163	-	-	260	-	-	89	-	-
	2e6 (high)	5c002-11f	12,198,873	-	-	31	22	57	-	-	43	-	-	30	774
		5c002-12f	13,472,951	-	-	-	-	-	-	-	-	-	107	-	447,260
		5c002-13f	24,826,217	-	-	-	-	-	-	-	-	-	-	-	-
		5c002-14f	24,878,733	26	1,523	-	-	1,361	36	-	-	-	-	537	-
		5c002-15f	14,471,936	91	-	-	-	-	1,752	-	-	628	-	21	87

The biodistribution and shedding of the CAL1 virus were assessed by measuring the A56R viral gene copies by qPCR. Data, expressed as copies per µg of DNA. Samples were collected from blood, urine, faeces and tissue samples on Days 16, 22 and 29 after last CLD-201 administration.

After intratumoral administration, data showed the virus was present mainly in the tumour and clearly replicates and accumulate within the tumor.

The absence of T cells can result in an impaired ability to clear viral infections

References and Acknowledgement :

- Minev BR et al. First-in-human study of TK-positive oncolytic vaccinia virus delivered by adipose stromal vascular fraction cells. J Transl Med. 2019 Aug 19;17(1):271. doi: 10.1186/s12967-019-2011-3.
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