CAL1 vaccinia virus as oncolytic agent and potential use of cell-based platform to enhance its therapeutic effects

Duong H. Nguyen[1], Thomas Herrmann[2], Forrest Neuharth[1], Ashley Alamillo[1], Barbara Haertl[2], Alberto Gomez[1], Ivelina Minev[1], Laura Edith Schneider[2], Boris Minev[1], Dobrin Draganov[1], Antonio F. Santidrian[1]

[1]Calidi Biotherapeutics, San Diego, CA 92121 I [2]StemVac, Bernried, Germany, D-82347

Background

Oncolytic virotherapy is a promising immuno-oncology approach that has not realized its potential due to rapid elimination by humoral immunity mediated by complement and neutralizing antibodies. We propose to use an adipose-derived mesenchymal stem cell-based platform, where the virus can be protected and amplified and potentiated inside the stem cells in order to minimize the clearance by anti-viral immunity. In this study, we evaluate the ability of CAL1 vaccinia virus (clonal derivative of Dryvax vaccine) to (1) selectively kill cancer cells, (2) to be genetically modified without affecting its natural tumor selectivity, and to (3) determine if a stem cell-based platform can protect the virus from inactivation and potentiate its anti-tumor effects.

Methods

CAL1 was tested for its ability to replicate and selectively kill various human prostate cancer cell lines in vitro and In Vivo. Additionally, CAL1 was loaded into adipose-derived mesenchymal stem cells to generate a new therapeutic agent called SuperNova1 (SNV1). Both CAL1 and SNV1 were tested for their ability to kill cancer cells in the presence of active complement and neutralizing antibodies in cell culture as well as in mice. Furthermore, CAL1 was used as the backbone to generate derivative CAL2 viruses using CRISPR/Cas9 technology to insert the gene encoding the fluorescent protein TurboFP into the intergenic locus between ORF-157 and ORF-158 of CAL1 without disrupting any existing CAL1 ORFs.

Results

We showed that in vitro CAL1 preferentially infected, amplified in and lysed tumor cells and was also able to cause tumor regression In Vivo without signs of toxicity. Furthermore, we demonstrated that the backbone of CAL1 can be used to engineer recombinant viruses, CAL2, that carry therapeutic genes without additionally attenuating the ability of the virus to amplify or kill tumor cells. SNV1 significantly enhanced protection of CAL1 virus from clearance by the immune system, leading to higher therapeutic efficacy. Furthermore, SNV1 provided instantly active viral particles for immediate infection and simultaneous release of therapeutic proteins in the injected tumors.

Conclusions

CAL1 could be used as an oncolytic agent. We show here that a major advantage of using a cell-based platform to deliver and potentiate oncolytic vaccinia virus is the prevention of viral inactivation by the humoral immune system resulting in enhanced oncolytic viral therapy.

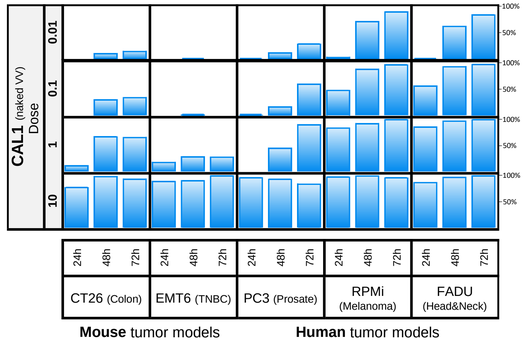
Graphic Abstract

Unprotected virus is quickly eliminated by the immune system before reaching the tumor cells

Calidi's Novel Treatment and Supernova Vaccinia for efficient delivery of oncolytic viruses

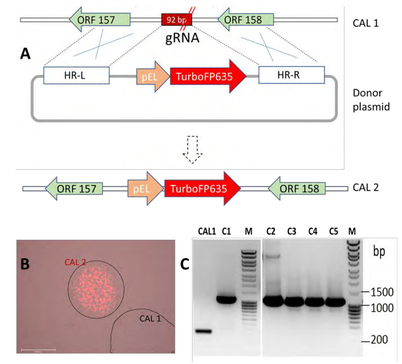


Oncolysis Of A Panel Of Tumor Cell Lines After Infection With Vaccinia Virus CAL1



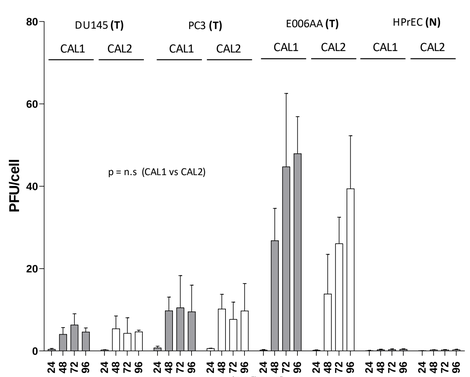
Oncolysis analysis of CAL1 virus on two mouse and three human cancer cell lines. Five indicated cell lines were seeded on 96-well electronic microtiter plates (E-Plate® 96). 24h later the cells were infected with CAL1 virus at different MOI of 0.01, 0.1, 1 or 10. xCELLigence Real-Time Cell Analysis (RTCA) was analyzed. Graph showed percentage (0-100%) oncolysis of CAL1-treated cell lines at 24h, 48h and 72h post virus infection (samples were analyzed in triplicate).

Genetically Engineering Of CAL2 Virus From Parental CAL1



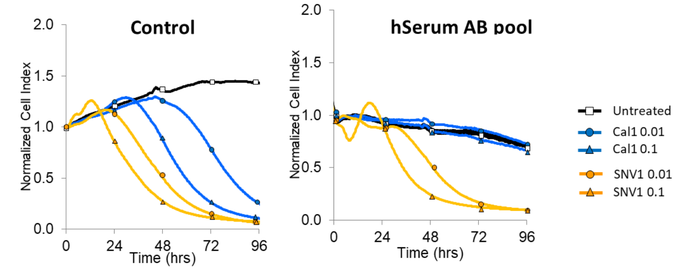
Generation of CAL2. (A) The intergenic locus in CAL1 genome was cut by Cas9HFc/gRNA, which trigger homologous recombination between CAL1 and the donor vector. TurboFP635 expression cassette from the donor vector was exchanged for the 92 bp fragment in the intergenic locus of CAL1, resulting in the recombinant virus, CAL2. **(B)** A mixture of recombinant (CAL2) and parental (CAL1) viruses were diluted and transfected on a monolayer of CV-1 cells. Clone selection of the recombinant CAL2 virus (red) was isolated from parental CAL1 virus (white). **(C)** Genomic DNA from selected clones were used as template for PCR. The recombinant clones (C1-C5) have a band with molecular weight higher than 1000 bp while the parental CAL1 virus is about 230 bp.

CAL1 And CAL2 Preferentially Amplifies In Tumor Cells



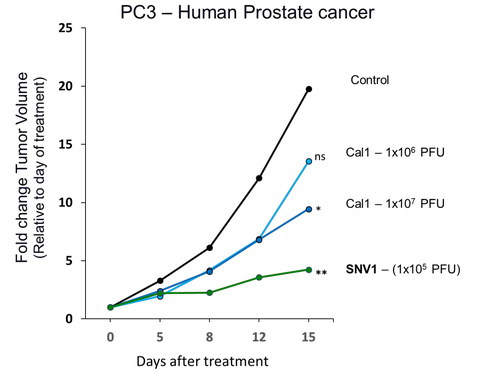
Comparison of CAL1 and CAL2 Viral Amplification. CAL1 and CAL2 preferentially amplified in prostate cancer derived human tumor cell lines PC3, DU145, and E006AA, but not in human primary prostate epithelial cell line HPrEC (MOI of 0.01). Samples were collected at 24, 48, 72, and 96 h post-infection, freeze and thawed, and used to infect CV-1 cells for 1-2 h. At 48 h post-infection plaques on CV-1 cells were quantified to determine viral amplification.

Ad-MSCs Protect Vaccinia Virus Against Human Serum-induced Inactivation



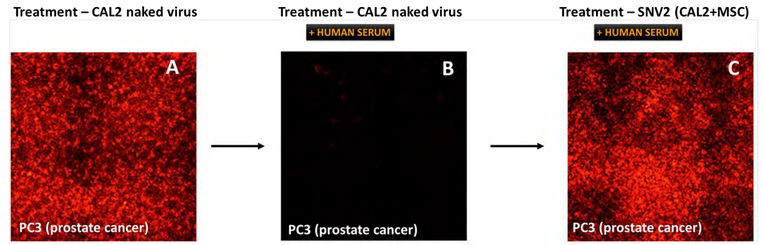
Cytolysis of PC3 human prostate cancer post infection with SNV1 or CAL1 virus. PC3 cancer cells were seeded a day before and infected with either naked CAL1 virus or SNV1 at Multiplicity of infection (MOI) of 0.1 or 0.01 in the presence of 20% human AB serum or FBS (control). PC3 killing (cytolysis) was automatically analyzed using xCELLigence Real Time Cell Analysis Instrument.

Ad-MSCs Increase Therapeutic Efficacy Of CAL1 In Vivo



Tumor growth inhibition of CAL1 and SNV1 in human prostate cancer model (PC3). Tumor bearing mice with volume around 100 mm^3 were I.T injected with 1 dose of CAL1 (10^6 and 10^7 pfu) or 10^6 cells incubated with 10^5 pfu CAL1 (SNV1) for 40 hours (5 pfu per 1 SNV1). 8 mice for each group. Groups were compared by unpaired two-tailed mann whitney test (ns: no significant *P < 0.05, **P < 0.01).

SNV Protects Vaccinia Virus Against Human Serum-induced Inactivation



Armed-viruses and their viral-encoding therapeutic proteins will only be expressed in tumor cells when properly protected and delivered by stem cells (SNV). Monolayer of human prostate cancer cells were infected with CAL2 or SNV2 at MOI of 1. (A) Naked CAL2 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 (CAL2). Pictures were captured 24h post virus infection.