A cell-based platform to potentiate oncolytic virus therapies

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Background

Oncolytic virotherapy has been pursued by multiple companies and institutions with few candidates reaching the clinic and demonstrating limited efficacy. The therapeutic potential of oncolytic viruses can be severely restricted by innate and adaptive immune barriers. To overcome this obstacle, we utilized adipose-derived stem cells (AD-MSC) loaded with tumor selective CAL1 oncolytic vaccinia virus to generate a new therapeutic agent called SNV1 (SuperNova-1).

Methods

SNV1 was generated by incubating AD-MSC with CAL1 virus. SNV1 was then analyzed for its ability to kill cancer cell lines and protect the oncolytic virus in the presence of active neutralizing antibodies and complement. In animals, SNV1 was injected intratumorally in various xenograft and syngeneic models. Immune cell infiltration of the injected tumors was analyzed by flow cytometry.

Results

Compared to the naked virus, SNV1 showed improved protection against the humoral barriers and efficient eradication of various human cancer cell lines *in vitro*. Intratumoral SNV1 treatment showed statistically significant tumor growth inhibition when compared to control non-treated tumors or to CAL1 naked virus treatment in all tested syngeneic tumor models (prostate, breast, melanoma, colon, and prostate cancers). Importantly, local administration of SNV1 induced systemic therapeutic effects. Five days after SNV1 administration, tumor infiltrating lymphocytes (TILs) from both treated and untreated tumors showed increased CD4 and CD8 T-cell infiltrations. Importantly, we documented a decreased frequency of Tregs, and improved effector to Treg ratios, which was associated with inhibition of tumor growth at the treated tumor site and also at distant untreated sites.

Conclusions

This study demonstrates the ability of our cell-based platform to protect and potentiate oncolytic vaccinia virus by circumventing humoral innate and adaptive immune barriers, resulting in enhanced oncolytic virotherapy. These findings provide fundamental rationale for the development of cell-based platforms to maximize the therapeutic potential of various oncolytic viruses.

Graphic Abstract

Scan me

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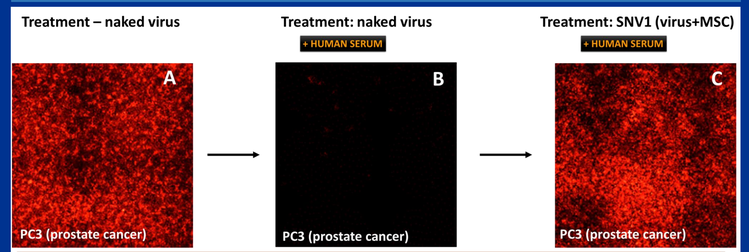
"SNV1 Cell-based platform protects and potentiates oncolytic vaccinia virus by circumventing humoral innate and adaptive immune barriers and modifying TME, thus resulting in enhanced oncolytic virotherapy"

SNV1 protects vaccinia virus against human serum-induced inactivation



CAL1 was engineered to express red fluorescent protein. RED signal indicates virus amplification and killing of cancer cells

% Cytolysis	Media	AB serum	Donor #1	Donor #2	Donor #3	Donor #4
Control	0	0	0	0	0	0
CAL1 0.1 (naked virus)	34	0	5	0	0	2
SNV1 0.1	80	72	63	60	25	27

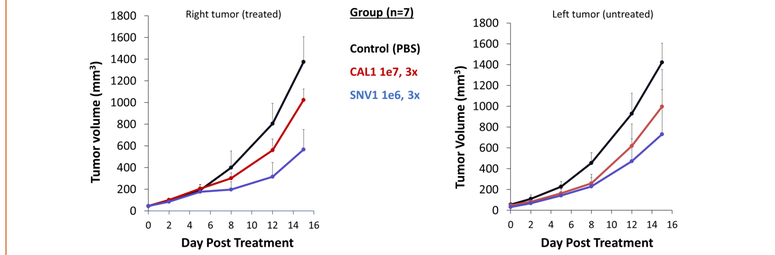
Percentage of cytolysis at 48 hours post infection with SNV1 or CAL1 virus. PC3 prostate cancer cells were seeded a day before and infected with either naked CAL1 virus or SNV1 at Multiplicity of infection (MOI) of 0.1 in the presence of different human sera. PC3 killing (cytolysis) was automatically analyzed using xCELLigence Real Time Cell Analysis Instruments.

SNV1 therapeutic responses in different tumor-bearing mouse models

Cell Line	Cancer Type	Day Post treatment	Growth Inhibition	Treatment	Model
B16-F10	Melanoma	11	**78%**	SNV1: 10^6	syngeneic
EMT-6	Breast	18	**69%**	SNV1: 10^6	syngeneic
CT-26	Colon	20	**77%**	SNV1: 10^6	syngeneic
Tramp-C2	Prostate	27	**99%**	SNV1: 10^6	syngeneic
RM-1	Prostate	7	**57%**	SNV1: 10^6	syngeneic
PC3	Prostate	14	**59%**	SNV1: 10^6	xenograft
MDA-MB-231	Breast	46	**98%**	SNV1: 10^6	xenograft

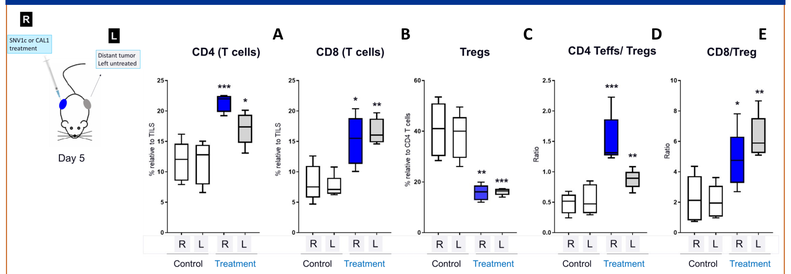
Tumor growth inhibition treated with 10^6 SNV1 in different mouse models. All indicated tumor cell lines were subcutaneously implanted in mice (either with immunocompetent or immunocompromised immune system) to form tumor. When the tumor volume reached around 60-150 mm^3, mice were treated intratumorally with SNV1, CAL1 or PBS control (n=7-10). Tumor volume was measured 3 times per week. Mice were sacrificed if the tumors were bigger than 2000 mm^3.

SNV1 inhibits treated and also distance untreated tumors



CT26 prostate tumor growth inhibition following treatment with CAL1 or SNV1. Animals were inoculated subcutaneously with 100 µL of CT26 cells (2.5 x 10^6 cells) in the right and left abdominal flank regions. Once tumors reached the volume about 60 mm^3, the animals were randomized and I.T. treated with PBS, 10^7 CAL1 naked virus, or 10^6 SNV1 three times every 2 days. Tumor volume was measured twice per week.

Local administration of SNV1c modulates local and distant tumor immune 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.