Background:
Different types of viruses, including vaccinia virus (VACV), can selectively target cancer cells and trigger antitumor immunity. Oncolytic virotherapies, as a monotherapy or in combination with other immunotherapeutics, have shown promising safety and proof-of-concept results in pre-clinical studies as well as in several clinical trials. The therapeutic potential of oncolytic viruses, however, can be severely restricted by multiple innate and adaptive immune barriers that can be overcome using cell-based delivery approaches. Mesenchymal stem cells are particularly attractive as carriers of oncolytic viruses due to their unique immunosuppressive properties that allow them to protect the virus from complement/antibody-mediated neutralization and to overcome anti-viral cellular immunity in both autologous and allogeneic settings.

Methods:
As carriers of oncolytic VACV, we used cells a) freshly isolated from adipose tissue stromal vascular fraction (SVF), and b) SVF-derived cultured Adipose-Derived Mesenchymal Stromal/stem Cells (AD-MSC). We analyzed the ability of these carrier cells to protect, deliver, and amplify the virus as well as to overcome innate and adaptive immune barriers by flow cytometry, microscopy and virus plaque assays of ex vivo co-cultures of cells infected with VACV in the presence of human serum or peripheral blood mononuclear cells from healthy donors. Comparative analysis was performed to establish statistically significant correlations and to evaluate the effect of stem cells on the activity of key immune cell populations.

Introduction

Calidi Biotherapeutics’ Therapeutic Platform

![Therapeutic Platform](image)

Calidi's Therapeutic Platform: Preclinical Regulatory Phase 1 Phase 1b/2

- CAL1 virus + autologous SVF
  - Completed Autologous proof of concept
  - Allogeneic - Off-the-shelf product
- CAL1 virus + allogeneic AD-MSC
  - Phase 1b/2 Q4-2019

1. Fresh SVF Overcomes Serum-Induced Inactivation of Vaccinia Virus

![Fresh SVF Overcomes Serum-Induced Inactivation of Vaccinia Virus](image)

- Fresh SVF, population rich in MSC precursors, can protect oncolytic viruses and dramatically increase therapeutic efficacy. Representative images of PC3 prostate cancer cells treated with a) CALL1-TurboFP35 (VACV Wyeth strain engineered to express red fluorescent turboFP35 protein for 24 hours) b) CALL1-TurboFP35 in presence of 20% Human serum or c) CALL1-TurboFP35 loaded in fresh SVF and in presence of 20% Human serum.

2. SVF Cell Populations, SA-ASC and Pericytes (AD-MSC precursors) Hide and Deliver CALL1 into Tumor Cell Monolayers

![SVF Cell Populations, SA-ASC and Pericytes (AD-MSC precursors) Hide and Deliver CALL1 into Tumor Cell Monolayers](image)

- SVF cell populations carrying CALL1. SVF from three different non-cancer patients were loaded with CALL1 during 1 hour at 37°C using a continuous rotation at 20 RPM. After CALL1 virus loading, SVF cells were labeled with a panel of antibodies, CD34a, CD45, CD14, CD31 and CD166 and stained for viability with PI. Populations were characterized and sorted as: Erythrocytes (CD205+), SA-ASC (CD105+CD45+CD34+CD14-CD31-), Pericytes (CD105+CD45-CD14+CD31+), Granulocytes, and Lymphocytes (CD105+CD45+CD31-), SSC High). Erythrocytes (CD205+), Erythrocytes (CD205-), Erythrocytes (CD205-), Erythrocytes (CD205-), Erythrocytes (CD205-), Erythrocytes (CD205-). Cells were characterized and sorted by flow cytometry using FACSCa Fusions. Sorted cells were then seeded into PC3 prostate tumor cell monolayers and % of infected sorted cells were analyzed by measuring plaque formation.

3. Cultured AD-MSCs Can Amplify Vaccinia Virus

![Cultured AD-MSCs Can Amplify Vaccinia Virus](image)

- The adipose-derived Mesenchymal stem cells (AD-MSC) can amplify 100-200 times vaccinia virus. Established adipose-derived stem cells (AD-MSC) cell lines derived from 4 different SVFs were incubated with VACV (MOI=0.01) for 48 hours. Recovered viruses (PFU/sample) were analyzed by plaque assay.

4. AD-MSCs Can Potentiate Oncolytic Virotherapy

![AD-MSCs Can Potentiate Oncolytic Virotherapy](image)

- Representative Images of PC3 prostate cancer cells treated with a) CALL1-TurboFP35 (VACV Wyeth strain engineered to express red fluorescent turboFP35 protein for 24 hours) b) CALL1-TurboFP35 in presence of 20% Human serum or c) CALL1-TurboFP35 loaded in culture AD-MSC and in presence of 20% Human serum.

5. AD-MSCs Enhance Oncolytic of Resistant Tumor Cells

![AD-MSCs Enhance Oncolytic of Resistant Tumor Cells](image)

- The adipose-derived stem cells (AD-MSCs) amplify oncolytic vaccinia virus and eradicate tumor cells resistant to virotherapy. (A) AD-MSCs amplify VACV to levels much higher than resistant (B16, mouse melanoma) and comparable to the most permissive tumor cell lines (LSA9 Human Lung cancer). (B) Image analysis of B16 melanoma cells infected with VACV TurboFP35-engineered vaccinia virus in the presence of GFP-labeled AD-MSC. (C) AD-MSCs potentiate virus-mediated oncolysis and successfully eradicate melanocytes of resistant B16 melanoma cells.

6. AD-MSCs Can Overcome Anti-Viral and Allogeneic Barriers to Potentiate Oncolytic Virotherapy

![AD-MSCs Can Overcome Anti-Viral and Allogeneic Barriers to Potentiate Oncolytic Virotherapy](image)

- Our findings indicate the feasibility to significantly potentiate oncolytic virotherapy by using either autologous or a more scalable off-the-shelf allogeneic cell-based delivery technology.

- Poster P609 contains more information on the Phase I trial using fresh autologous SVF to potentiate Vaccinia Virus.

7. CONCLUSIONS

- Our findings indicate the feasibility to significantly potentiate oncolytic virotherapy by using either autologous or a more scalable off-the-shelf allogeneic cell-based delivery technology.

- Poster P609 contains more information on the Phase I trial using fresh autologous SVF to potentiate Vaccinia Virus.