Subcutaneous Intratumoral Delivery of CF33-hNIS-antiPDL1 Eradicates Distant Peritoneal Tumors

[2], Jianming Lu[1], Sang-In Kim[1], Susanne G. Warner[1,2], Yuman Fong[1], Yanghee Woo (ywhoo@coh.org) [1,2]

[1]Department of Surgery, City of Hope National Medical Center, Duarte, CA;
[2]Cancer Immunotherapeutics Program, Beckman Research Institute, City of Hope National Medical Center, Duarte, CA

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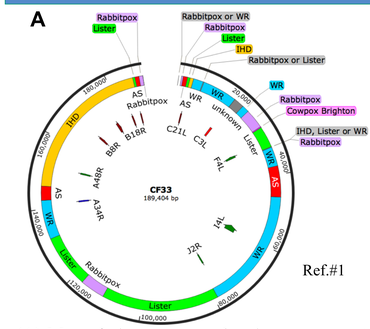
INTRODUCTION

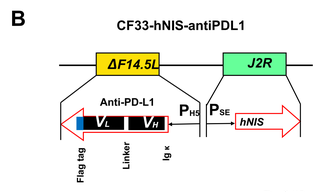
Oncolytic viruses (OVs) are therapeutic agents that have potent, cancer cell-specific antitumor activity, and can be used for targeted gene delivery. We have previously demonstrated that unmodified CF33 is safe, well-tolerated, and effectively kills pancreas cancer at doses of several magnitudes lower than other OVs currently under investigation. We engineered, CF33-hNIS-antiPDL1, on the backbone of CF33, a unique chimeric orthopoxvirus, which shows robust preclinical activity against many solid tumors and inherent strong anti-cancer activity against pancreatic ductal adenocarcinoma (PDAC). We investigated CF33-hNIS-antiPDL1 for its ability to track and kill distant peritoneal metastases after local virus administration in vivo. We show that subcutaneous intratumoral (SC.IT) delivery of CF33-hNIS-antiPDL1 decreases peritoneal tumor burden and improves survival in a PDAC mouse model.

METHODS

Oncolytic virus: We engineered CF33, a replication-competent orthopoxviral chimera to express the human sodium iodide symporter (hNIS) and anti-human programmed death-ligand 1 (anti-PD-L1) antibody to generate - CF33-hNIS-antiPDL1 (Schema 1). This third-generation CF33 variant was designed to synergize OV therapy with virus imaging/^{131}I treatment as well as enhance local anti-tumor immunity by blocking PD-1/PD-L1 interaction. ***Animal model***: To create our mouse model, we inoculated the human PDAC cell line - AsPC1-ffluc (5x10^6 cells/site) in both the subcutaneous (SC) space and the peritoneum in nude mice. ***Treatment:*** After confirming the presence of a tumor, the mice were divided into two groups and treated with either 1) three doses of SC intratumoral (SC.IT) injection of CF33-hNIS-antiPDL1 (3x10^5 plaque-forming units) or 2) PBS as control. ***Endpoints:*** Mice were assessed for: either tumor burden (bioluminescence imaging) and survival (time to death) or CF33-hNIS-antiPDL1 tracking and replication in tumors (^{124}I based PET/CT imaging) at one week.

Schema 1. Genomic analysis of CF33 and construction of CF33-hNIS-antiPDL1





(A) Map of virus genome showing components of parental viruses**.** Origin of genes involved in host immune-modulation (C21L, C3L, B8R, and B18R) and nucleotide metabolism (F4L, I4L, J2R, and A48R) are also shown (**B**) Schematic representation of the anti-PD-L1 scFv structure in virus sequence location.

Ref. #1. Chaurasiya S, Chen NG, Lu J, et al. A chimeric poxvirus with J2R (thymidine kinase) deletion shows safety and anti-tumor activity in lung cancer models. Cancer Gene Ther. 2020 Apr;27(3-4):125-135.

Ref. #2. Woo Y, Zhang Z, Yang A, et al. Novel chimeric immuno-oncolytic virus CF33-hNIS-antiPDL1 for the treatment of pancreatic cancer. J Am Coll Surg. 2020 Apr;230(4):709-717.

RESULTS

By week 2, animals in the CF33-hNIS-antiPDL1-treated group had significantly decreased tumor burden both in the peritoneal tumors (2.5e9±2.498e9 %/ID/cc versus 1.6e10 ±1.3e10 %/ID/cc, respectively; p<0.05) and SC tumors (5.2e8 ±4.3e8 %/ID/cc versus 2.6e9 ±2.6e9 %/ID/cc; p<0.05) compared to the PBS-treated group. All PBS-treated mice died from the disease between 21 to 47 days with a median survival of 35 days. Survival was prolonged in 2 out of 8 mice with complete tumor regression of both SC tumor and peritoneal carcinomatosis (p<0.05) in the CF33-hNIS-antiPDL1 treated group. Significantly increased PET/CT avidity for ^{124}I uptake in SC and peritoneal tumors was visible at day 7, following the first administration of CF33-hNIS-antiPDL1 as compared with the PBS-treated group.

RESULTS

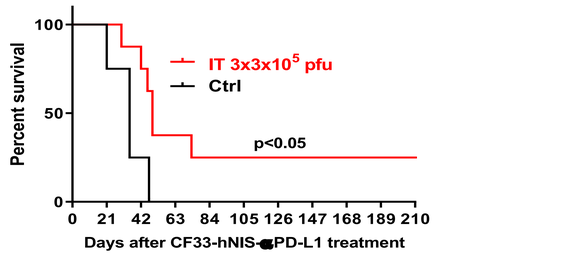


Figure 1. CF33-hNIS-antiPDL1 increases survival in nude mouse model of human PDAC AsPC-1flucc. Kaplan-Meier survival analysis of the experiment outlined in Figure 2A. PBS (n=4) and CF33-hNIS-antiPDL1 (n=8).

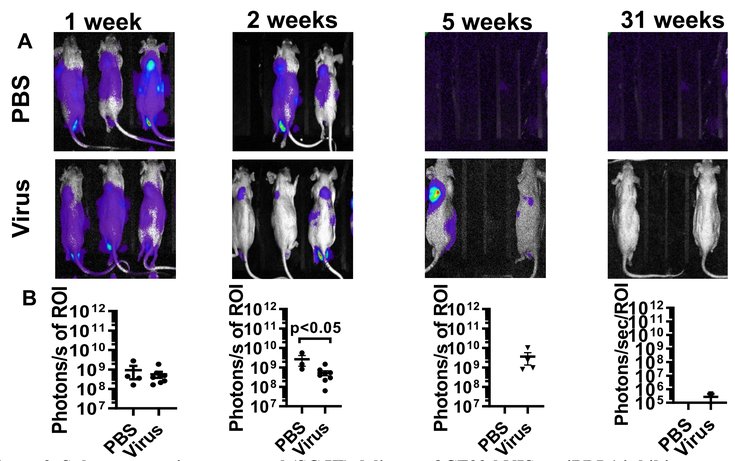


Figure 2. Subcutaneous intra-tumoral (SC.IT) delivery of CF33-hNIS-antiPDL1 inhibits subcutaneous tumor development. (**A**) Bioluminescence images of three representative SC.IT mice in each treatment group (**B**) Statistical analysis of bioluminescence imaging.

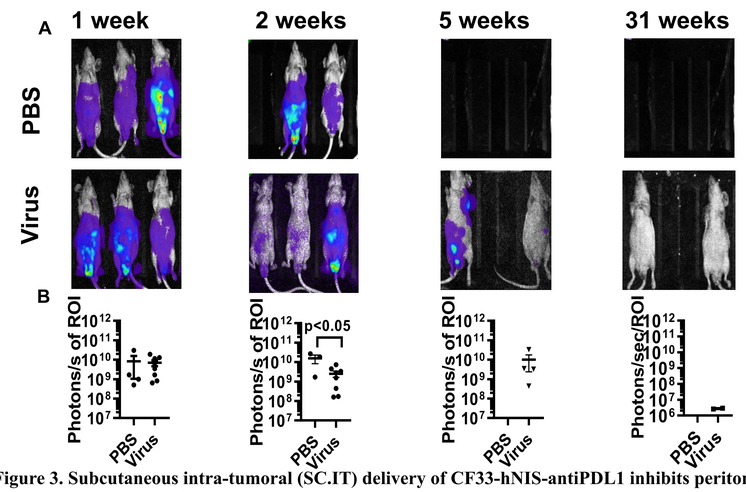


Figure 3. Subcutaneous intra-tumoral (SC.IT) delivery of CF33-hNIS-antiPDL1 inhibits peritoneal tumor development. ((**A**) Representative bioluminescence images of three mice with peritoneal tumors in each treatment group (**B**) Statistics analysis of bioluminescence imaging.

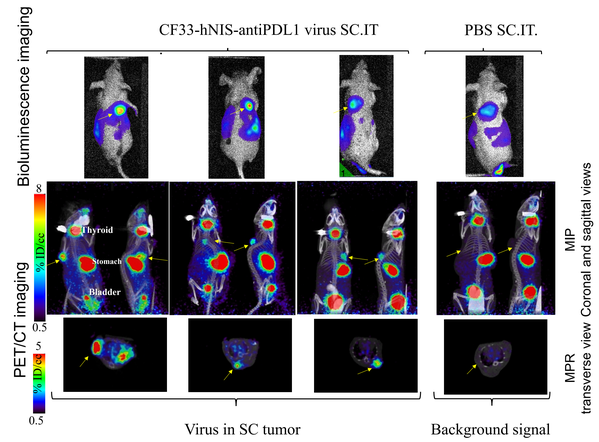


Figure 4. ^{124}I PET/CT imaging of the PDAC mouse model can monitor subcutaneous tumor burden. After SC.IT delivery of CF33-hNIS-antiPDL1, virus-encoded hNIS are expressed in tumor cells. On day 7, PET/CT images of virus S.C. IT group and PBS group were shown after i.v. injection of 200 mCi of ^{124}I for 2 hours . Note: Thyroid and stomach has endogenous hNIS expressions intensively avid on PET.

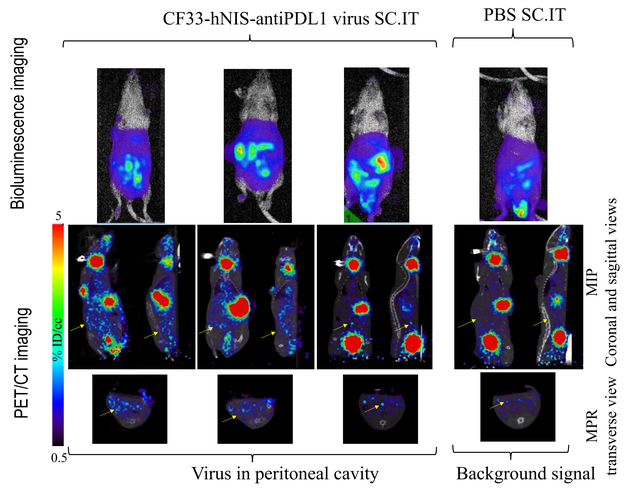


Figure 5. ^{124}I PET/CT after SC.IT injection of CF33-hNIS-antiPDL1 identifies tumor nodules in the peritoneum. PET/CT images of peritoneal cavity at day 7 after S.C.IT CF33-hNIS-anti-PDL1 injection versus PBS are shown with i.v. injection of 200 mCi of ^{124}I for 2 hours.

CONCLUSION

Local SC.IT administration of CF33-hNIS-antiPDL1 efficiently kills local tumor and distant peritoneal metastases to improve survival. ^{124}I-based PET/CT imaging can be used to visualize sc and peritoneal tumors treated with CF33-hNIS-antiPDL1.

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