Novel Chimeric Poxvirus CF17 Improves Survival in a Murine Model of Intraperitoneal Ovarian Cancer Metastasis

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INTRODUCTION

Despite improvements in surgical techniques and chemotherapy, ovarian cancer remains the most lethal gynecologic cancer. Thus, there is an urgent need for more effective therapeutics, particularly for chemo-resistant peritoneal ovarian cancer metastases. Oncolytic virotherapy represents an innovative treatment paradigm; however, for oncolytic viruses tested from the last generation of genetically engineered viruses, the therapeutic benefits have been modest. To overcome these limitations, we generated a chimeric poxvirus, CF17, through the chimerization of nine species of orthopoxviruses. Compared with its parental viruses, CF17 has demonstrated superior oncolytic characteristics. Here, we report the oncolytic potential of CF17 in ovarian cancer. Replication of CF17 and its resulting cytotoxicity were observed at multiplicities of infection (MOIs) as low as 0.001 in human and mouse cancer cell lines in vitro. Furthermore, CF17 exerted potent antitumor effects in a syngeneic mouse model of ovarian cancer at doses as low as 6 × 10^6 plaque-forming units. Together, these data merit further investigation of the potential use of this novel chimeric poxvirus as an effective treatment for aggressive intraperitoneal ovarian cancer.

RESULTS

CF17 Infects and Replicates in Human and Mouse Ovarian Cancer Cell Lines In Vitro

We infected murine (ID8) and human (OVCAR8, SKOV3) ovarian cancer cells with CF17 at various multiplicities of infection (MOIs). We performed an 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay to analyze tumor cell viability 3 days post-CF17 infection. For all cell types tested, non-infected cells were used as negative control. We observed that infection with CF17 eliminated these cancer cells at an MOI of 10.
CF17 demonstrates significant antitumor activity in a syngeneic mouse model of ovarian cancer

CF17 treatment improves survival and reduces ascites in a syngeneic mouse model of ovarian cancer

DISCUSSION

Studies have shown that oncolytic virotherapy is clinically safe and non-toxic in different cancer patients. Despite their high safety profiles, the last generation of OVs has shown limited efficacy. Oncolytic poxviruses have demonstrated encouraging results in multiple preclinical tumor models, as well as some clinical trials for the treatment of various cancers.
only two injections of $6 \times 10^6$ PFUs, at a dose far lower than that commonly reported for other oncolytic poxviruses.\textsuperscript{29–32}

In summary, CF17 is safe and exerts antitumor effects against human ovarian cancer \textit{in vitro} and murine ovarian cancer \textit{in vivo}. Further studies are needed to determine whether CF17 can be used either as a monotherapy or in combination with compatible therapeutics. The data presented here warrant further testing of CF17 for clinical use.

MATERIALS AND METHODS

\textbf{Generation of CF17 nChimeric Virus}

To generate CF17, we used nine strains of orthopoxvirus in co-infecting CV-1 cells and fostering chimerization. These included raccoon-pox virus strain Herman, cowpox virus strain Brighton, rabbitpox virus strain Utrecht, and vaccinia virus strains AS, Connaught Laboratories, Elstree, IHD, Lederle-Chorioallantoic, and Western Reserve, all purchased from ATCC. Following the chimerization, 100 individual plaques were chosen and then purified through three rounds of plaque purification to obtain 100 clonally purified chimeric orthopoxviruses. High-throughput screening was used to compare the cytotoxic efficacy against the NCI-60 panel. CF17 was selected as a chimeric isolate, which demonstrated superior cell killing in the NCI-60 panel when compared with all parental viruses.

\textbf{Cell Culture}

The OVCAR8 human ovarian cancer cell line was generously provided by Dr. Carlotta Glackin (City of Hope). The SKOV3 human ovarian cancer cell line was obtained from ATCC. The ID8 murine ovarian line was obtained from Dr. Katherine Roby (University of Kansas) and then transduced to express firefly luciferase. Ovarian cancer cell lines were cultured in RPMI basal media with 10% fetal bovine serum (Gemini Bio), 1% L-glutamine (Invitrogen), and 1% penicillin-streptomycin (Invitrogen). Cells were maintained at 37°C in a humidified incubator (Thermo Electron Corporation) containing 6% CO$_2$ and then were harvested. For all cell lines, when cells reached 80% confluency, they were passaged using 0.25% trypsin and EDTA solution (Invitrogen); media were changed every 2–3 days.

\textbf{Virus Proliferation and Cytotoxicity Assays}

To determine the ability of viruses to replicate in cultured cells, we infected cells at an MOI of 0.03, and virus titers in the lysates were determined using a standard plaque assay, as described previously.\textsuperscript{18} A CellTiter 96 AQueous colorimetric assay (Promega) was used to measure cell survival after viral infection. In brief, cells were infected in 96-well plates at various MOIs (0.001–10), the substrate was added 72 h post-infection, and absorbance was measured at 490 nm using a plate reader (Tecan Spark). The survival of CF17-treated cells was calculated relative to that of non-infected control cells.

\textbf{In Vivo Efficacy of CF17 in an Orthotopic Ovarian Cancer Model}

All animal experiments were conducted in accordance with NIH Guidelines for the Care and Use of Laboratory Animals and City of Hope regulations after review and approval by the City of Hope Institutional Animal Care and Use Committee (protocol #18002).
Female C57BL/6J mice (6–8 weeks old; The Jackson Laboratory) were inoculated with $5 \times 10^6$ firefly luciferase-labeled ID8 cells via i.p. injection. At 1 and 7 days post-inoculation, mice were administered i.p. $6 \times 10^6$ PFUs of CF17 (n = 4) or vehicle (n = 3). Bioluminescence imaging (BLI) was performed once a week after tumor implantation using the Lago imaging system (Spectral Instruments Imaging). Prior to imaging, mice were injected i.p. with D-luciferin (Xenogen; 150 μL/mouse). Anesthesia was induced with 2% isoflurane (Abbott Laboratories) in a transparent airtight box for 5–7 min before the mice were moved to the light-tight chamber of the charge-coupled device (CCD) camera in the imaging position. The images were analyzed using Aura software version 2.2.0 (Spectral Instruments Imaging).

**Statistical Analysis**

Data are presented as mean ± SEM unless otherwise stated. Statistical significance for tumor flux at each time point between the groups was examined using one-way ANOVA ($p < 0.05$ was deemed to be significant). Survival analysis was carried out using Kaplan-Meier curves. The survival distributions of vehicle- and CF17-treated mice were compared using log-rank tests.

**AUTHOR CONTRIBUTIONS**

Conceptualization, K.S.A., M.H., T.H.D., N.G.C.; Methodology, M.H., Y.C.; Formal Analysis, M.H., M.L.; Investigation, M.H., Y.C., J.L.; Writing – Original Draft, M.H.; Writing – Review & Editing, M.H., K.S.A., Y.F., R.M.; Visualization: M.H., Y.C., K.S.A.; Funding Acquisition, M.H., Y.C., K.S.A.; Resources, Y.F., K.S.A.; Supervision, K.S.A., Y.F.

**CONFLICTS OF INTEREST**

K.S.A. is CSO with an advisory role and stock ownership in TheraBiologics, Inc. All other authors declare no competing interests.

**ACKNOWLEDGMENTS**

We would like to acknowledge the essential contribution of Shyam-babu Chaurasiya, PhD, for helping to design the in vitro CF17 experiments. We are grateful to Supriya Deshpande, PhD, for editing the manuscript. This work was funded by STOP CANCER, the Rosalinde and Arthur Gilbert Foundation, the California Institute of Regenerative Medicine, the Alvarez Family Foundation, the Anthony F. & Susan M. Markel Foundation, the Ben and Catherine Ivy Foundation, City of Hope, and National Cancer Institute grants R43CA86768, R44CA8678, and R01CA197359. Research reported in this publication includes work performed by M.L. in the City of Hope Biostatistics Core, which is supported by the National Cancer Institute of the NIH under award number P30CA033572. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Materials Transfer Information is available from the City of Hope Office of Technology Licensing. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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