The Use of a Tropism-Modified Measles Virus in Folate Receptor–Targeted Virotherapy of Ovarian Cancer

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Abstract

Purpose: Attenuated measles viruses are promising experimental anticancer agents currently being evaluated in a phase I dose escalation trial for ovarian cancer patients. Virus attachment, entry, and subsequent intercellular fusion between infected and uninfected neighboring cells are mediated via the two measles receptors (CD46 and SLAM). To minimize potential toxicity due to measles virus–associated immunosuppression and infection of nontarget tissues, we sought to develop an ovarian cancer exclusive fully retargeted measles virus.

Experimental Design and Results: Interactions of measles virus with its natural receptors were ablated, and a single-chain antibody (scFv) specific for α-folate receptor (FRα), a target overexpressed on 90% of nonmucinous ovarian cancer, was genetically engineered on the viral attachment protein (MV-αFR). Specificity of virus tropism was tested on tumor and normal cells. Biodistribution of measles virus infection was evaluated in measles-susceptible CD46 transgenic mice, whereas antitumor activity was monitored noninvasively by bioluminescence imaging in xenograft models. Tropism and fusogenic activity of MV-αFR was redirected exclusively to FRα without compromise to virus infectivity. In contrast to the parental virus, MV-αFR has no background infectivity on normal human cells. The antitumor activity of MV-αFR, as assessed by tumor volume reduction and overall survival increase, was equal to the parental virus in two models of human ovarian cancer (s.c. and i.p.).

Conclusions: A FR-exclusive ovarian cancer targeted oncolytic virus was generated and shown to be therapeutically effective, thus introducing a new modality for FR targeting and a candidate measles virus for clinical testing.

Live attenuated measles virus has promising oncolytic activity against a variety of tumor cells and xenografts (1, 2). Two recombinant measles viruses engineered for noninvasive monitoring of the pharmacokinetics of viral gene expression through the use of a soluble marker peptide (MV-CEA) or the human thyroidal sodium iodide symporter (MV-NIS) are being tested in phase I clinical trials for patients with recurrent ovarian cancer, glioblastoma, and multiple myeloma (3–5). Measles virus uses its coat protein, hemagglutinin (H), to attach to one of two viral receptors, CD46 or SLAM (signaling lymphocyte activation molecule), and the fusion (F) protein to mediate virus entry and subsequent virus spread by cell-to-cell fusion (6, 7). Thus, a unique feature of measles virus tumor cell killing is an extensive cytopathic effect of syncytial formation, which, in addition to viral replication, significantly increases bystander killing of neighboring cells by the agent (1, 8).

To minimize virus sequestration by non-target cells and collateral damage to normal tissues, the tropism and cytopathic activity of an oncolytic virus should ideally be restricted to tumor cells. The measles CD46 receptor, a regulator of complement activation, is ubiquitously expressed on all nucleated human cells, whereas SLAM is expressed on immune cells (7, 9). The characteristic immunosuppression associated with measles infection is thought to be due to interaction of measles H protein with CD46 and/or SLAM (10, 11). Hence, we recently engineered mutations in the H protein to ablate virus interactions with its native receptors and established a virus rescue system using a pseudoreceptor (His-6 tag) that allowed rescue and propagation of CD46/SLAM blind, fully retargeted measles viruses displaying scFv against CD38 (MV-αCD38) and the epidermal growth factor receptor (EGFR), MV-αEGFR (12).

Epithelial ovarian cancer is the leading cause of gynecologic cancer death in the United States, but neither MV-αCD38 nor MV-αEGFR is ideal for ovarian cancer therapy (13). EGFR is a nonspecific target as it is expressed on most epithelial cells,

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whereas CD38, a plasma cell marker, is not found on ovarian cancer cells. Among the membrane-associated targets, the α-folate receptor (FRα) is highly promising (14, 15). Elevated expression of FRα has been observed in various types of cancers, including ovarian, uterine, endometrial carcinoma, and pleural mesothelioma (14, 16–19). FRα expression in normal tissues is restricted to the apical surfaces of polarized epithelial cells where it is inaccessible to circulating cytotoxic drugs (14, 15, 17). Hence, there is much interest to use FR as target for tumor-specific killing using various types of anticancer therapeutics but none yet with a replicating oncolytic virus (14, 20).

In the current study, we generated a tropism modified measles virus displaying a FRα-specific scFv derived from MOv18, a monoclonal antibody that has been extensively tested in ovarian cancer clinical trials (21–23). The FRα-targeted measles virus can infect and destroy FRα-positive tumors efficiently and exclusively via the displayed scFv, warranting further investigation as a retargeted measles virus for clinical testing.

Materials and Methods

Cell culture. Cell lines were purchased from the American Type Culture Collection (Manassas, VA) or have been described previously (12, 24–27). Peripheral blood lymphocytes from healthy volunteers were stimulated with 5 μg/mL phytohemagglutinin for 6 days before use. A375-FR, ARH-77-FR, CHO-FR, and SKOV3ip.1-Fluc-βhCG cells were generated by transduction of parental cells using VSV-G pseudotyped lentiviral vectors. To generate the lentivectors, 293T cells were cotransfected with gag-pol expression plasmid pCMVΔ8.91, VSV.G envelope expression plasmid pMD-G, and vector plasmid pHR-SIN-dlNotI encoding cDNAs for expression of FRα, firefly luciferase (Fluc), or the β chain of human chorinoic gonadotropin (βhCG; ref. 28). Supernatant was collected 48 hours later and frozen at −80°C.

Flow cytometry. Cells were incubated for 30 minutes on ice with MOv18 (a mouse anti-FRα monoclonal antibody at 1:100 dilution), washed twice, and incubated for 30 minutes with 1:150 dilution of FITC-conjugated goat anti mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA). After washing, the cells were fixed and analyzed by flow cytometry using FACScan with CellQuest software (Becton Dickinson, San Jose, CA).

Generation of FRα-retargeted measles virus. The cDNA for MOv18, an anti-FRα scFv (kindly provided by Dr. Y. Takeuchi, UCL, London), was PCR amplified as an *Sfi*I/*Not*I fragment and inserted in-frame into pTNH6aa, a shuttle vector encoding measles H and containing alanine substitutions at residues 481 and 533 (Fig. 1A). The *Pac*I/*Spe*I fragment was then inserted into p(+)MV-eGFP. For rescue of FRα-retargeted virus, the Six-his Tagging and Retargeting system was used (12). Virus stocks were harvested after infection of Vero-αHis cells at a multiplicity of infection of 0.02, and cell-associated viruses were harvested by freeze-thaw cycles. Virus stocks were titrated by 50% tissue culture infective dose ($TCID_{50}$) assay on Vero-αHis cells.

Immunoblotting. Viral samples (10^5 $TCID_{50}$; virus titer determined on Vero-His cells) were directly mixed with an equal volume of SDS loading buffer [130 mmol/L Tris (pH 6.8), 20% glycerol, 10% SDS, 0.02% bromophenol blue, 100 mmol/L DTT]. These samples were denatured for 5 minutes at 95°C, fractionated on a 7.5% SDS-polyacrylamide gel, blotted to nitrocellulose membrane (Amersham, Piscataway, NJ), and immunoblotted with anti-measles N and H protein as described previously (12).

Virus titration and infection. To compare growth characteristics of the recombinant viruses, Vero-αHis cells were infected with the viruses at a multiplicity of infection of 3.0 for 2 hours at 37°C. The inoculum was removed; the standard medium was replaced; and the cells were maintained at 32°C. At 12, 24, 36, 48, and 72 hours after infection, cells were scraped into 1 mL Opti-MEM (Life Technologies, Rockville, MD), and cell-associated viruses were released by two freeze-thaw cycles. Viral titers were determined by $TCID_{50}$ titration on Vero-αHis cells. For virus infection, cells (10^5 adherent cells or 10^6 suspension cells) were incubated with virus at a multiplicity of infection of 0.5 for 3 hours at 37°C. At 48 hours after infection, cells were photographed under fluorescence microscopy, and cell viability was determined by trypan blue exclusion assay.

In vivo ***experiments and noninvasive monitoring of tumor burden.*** All procedures involving animals were approved by and done according to guidelines of the Institutional Animal Care and Use Committee of Mayo Foundation. For *in vivo* targeting experiments, 2×10^6 ARH-77 or ARH-77-FR cells were implanted s.c. in the right flank of irradiated severe combined immunodeficient mice (150 cGy). When the tumors reached 0.5 cm in diameter, two doses of 2×10^6 $TCID_{50}$ MV-GFP, MV-αFR, or MV-αEGFR ($n = 3$ per group) were injected i.v. at 2 days apart. Four days after the last injection, mice were euthanized, and tumors were examined under fluorescence microscopy. For virus biodistribution analysis, 7- to 9-week-old measles susceptible Ifnar-CD46Ge transgenic mice (29) were given 1 dose of 2×10^6 $TCID_{50}$ MV-GFP or MV-αFR i.p. ($n = 3$ per group). Mice were euthanized 48 hours later and analyzed as described previously (30). For the therapy experiments, 2×10^6 SKOV3ip.1 cells were implanted s.c. in the right flank of female athymic mice (5-6 weeks of age; Taconic Laboratory, Germantown, NY). When the tumors reached 0.5 cm in diameter, mice received i.t. injections of MV-GFP ($n = 10$ mice per group), MV-αFR ($n = 10$) at 5×10^5 $TCID_{50}$ in 100 μL Opti-MEM, or vehicle (saline) control ($n = 9$), every other day for a total four doses (total dose = 2×10^6 $TCID_{50}$). In the i.p. ovarian cancer model, SKOV3ip.1 cells stably expressing Fluc and βhCG were used to enable noninvasive monitoring of tumor burden during the course of virotherapy. Mice were implanted i.p. with 2×10^6 SKOV3ip.1-Fluc-βhCG cells. Six days later, mice were received six doses (given every other days) of MV-GFP ($n = 10$), MV-αFR ($n = 10$) at 10^6 TCID50 in 500 μL Opti-MEM, or saline ($n = 9$) i.p. To monitor tumor burden, cohorts of five mice were bled for βhCG measurements and imaged using the IVIS 200 Bioluminescence Imaging System (Xenogen Corp., Alameda, CA). Plasma βhCG analysis was done by Mayo Clinic Central Clinical Laboratory. For imaging, mice were given i.p. injections of 150 mg/kg D-luciferin (Xenogen) 10 minutes before imaging. To quantitate tumor burden, whole abdominal bioluminescence signals were calculated from the imaging data using the Living Image software (Xenogen) according to manufacturer's protocol.

Statistical analysis. The differences in tumor burden (tumor volume, photon counts, and plasma βhCG) in each group were analyzed by two-way repeated measures ANOVA. Survival curves were represented using the Kaplan-Meier method. The log-rank test was used to examine the significance of differences in the survival between groups. We used GraphPad Prism (GraphPad Software, San Diego, CA) for the statistical calculations. $P < 0.05$ was considered significant.

Results

Generation and characterization of FRα-retargeted measles virus. The CD46/SLAM blind MV-αFR (Fig. 1A) was rescued and propagated via H6 peptide binding to its pseudoreceptor on Vero-αHis cells as described previously (12). Correct incorporation of the scFv on H was determined by immunoblotting of virions using an anti-measles H antibody. The chimeric H glycoprotein of MV-αFR showed a higher apparent

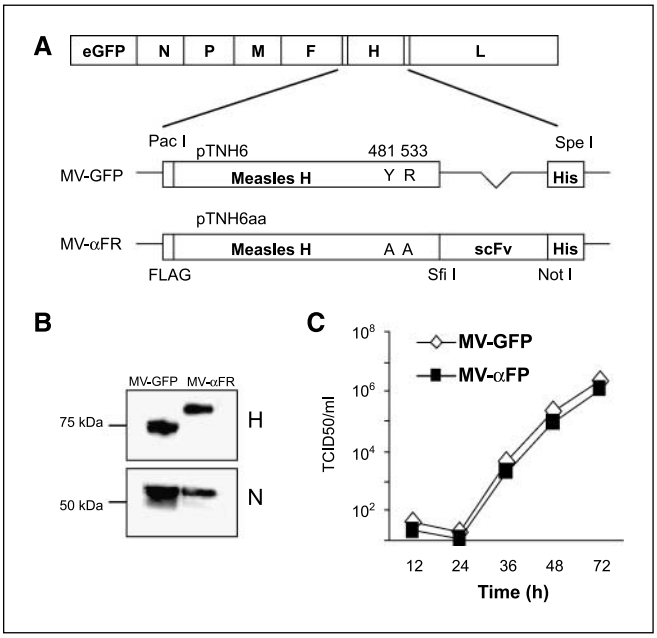

Fig. 1. Generation and characterization of the FRα-targeted measles virus. *A,* schematic representation of the unmodified (MV-GFP) and tropism-modified (MV-αFR) measles virus genomes. Mutations in H at 481Y → A and 533R → A ablate CD46/SLAM interaction. A single-chain antibody (scFv) is displayed as a COOH-terminal extension of mutated H protein followed by a six-histidine peptide (H6). *B,* immunoblot of the parental and chimeric virions using anti-H and anti-N antibodies. Equal titers of each virus were loaded. The chimeric H glycoprotein of MV-αFR (*lane 2*) has a higher molecular weight compared with that of MV-GFP (*lane 1*). *C,* growth kinetics of MV-αFR and MV-GFP on Vero-αHis cells were comparable.

molecular weight than the unmodified H (Fig. 1B). Replication kinetics of MV-αFR was compared with the MV-GFP parental virus in Vero-αHis producer cells. The one-step growth curves of both viruses were comparable (Fig. 1C), and viral titer stocks of MV-eGFP and MV-αFR were in the range of 3×10^7 and 1×10^7 $TCID_{50}$, respectively.

Specificity of MV-αFR infection and cytopathic effects were investigated on a panel of Chinese hamster ovary (CHO) cells expressing the respective receptors, CD46, SLAM, FRα, and CD38. To control for specificity of the αFR scFv, a recombinant measles virus displaying a scFv against CD38, a plasma cell marker, was used (MV-αCD38). As shown in Fig. 2, CHO cells expressing either CD46 or SLAM were infected by MV-GFP but not by the double-blind fully retargeted MV-αFR or MV-αCD38. FRα-positive CHO cells were infected efficiently by MV-αFR but not by MV-αCD38 that displays an irrelevant scFv and vice versa on CHO-CD38 cells. These data showed that MV-αFR was ablated for infection and cell fusion via both of the native measles virus receptors and is highly specific for FRα. Importantly, the one-step growth kinetics and final titers reached by the fully retargeted virus were not significantly compromised by displayed scFv and compared favorably with the parental virus.

In vitro *tumor-selective killing by FRα-retargeted virus.* Specificity of infection and cell killing by MV-αFR was investigated on a panel of human cancer cell lines and normal cells. The presence or absence of αFR receptor on these cells was analyzed by flow cytometry (Supplementary Figure). Cells were infected at a multiplicity of infection of 0.5, and after 48 hours, the presence of green fluorescent protein (GFP)–positive syncytia were noted, and numbers of viable cells were counted by trypan blue exclusion. MV-αFR caused cytopathic damage of extensive cell fusion in ovarian and breast cancer cell lines and not in normal cells and other cancer cell lines (Fig. 3A). In contrast, MV-GFP infected both tumor and normal cells, although the cytopathic damage induced in normal cells was minimal, with significantly fewer and smaller (4-5 nuclei) syncytia (Fig. 3A). These observed cytopathic effects correlated with tumor cell killing as determined by trypan blue exclusion (Fig. 3B). To confirm that infection and cell fusion were mediated specifically via FRα, we used IGROV1-DM99 cells stably transfected with a plasmid encoding a FRα-specific intrabody (MOv19) to knock down FRα expression (27) and also generated A375 cells stably expressing FRα (A375-FR) by lentiviral transduction. As shown in Fig. 3C and D, MV-αFR efficiently infected and caused massive cell fusion in FRα-positive IGROV1 and A375-FR but not in FRα-negative A375 and IGROV1-DM99.

Specificity of* in vivo *tumor targeting by MV-αFR. The *in vivo* targeting specificity of MV-αFR was evaluated in severe combined immunodeficient mice bearing s.c. ARH-77 or ARH-77-FR xenografts. ARH-77 cells express CD46 and SLAM but not FRα or EGFR. MV-GFP and measles viruses retargeted to FRα or EGFR were given i.v. to the mice. Four days after the last injection, tumors were harvested and examined under white light or fluorescence microscopy. As shown in Fig. 4A, both ARH-77 and ARH-77-FR xenografts were infected by the parental untargeted MV-GFP virus. In contrast, MV-αFR infected only the FRα-expressing ARH-77-FR xenografts. MV-αEGFR, which displays a scFv against EGFR, could not infect ARH-77 or ARH-77-FR xenografts.

Biodistribution of MV-αFR infection in measles-susceptible transgenic mice. Ifnar-CD46Ge transgenic mice, which express the human CD46 receptor with the same tissue specificity and whose type I IFN receptors were inactivated to facilitate virus spread (29, 30), were used to evaluate the tropism of the MV-αFR virus versus the parental CD46-tropic MV-GFP. Mice were injected i.p. with 2×10^6 $TCID_{50}$ MV-GFP or MV-αFR, and 48 hours later, mice were euthanized and examined under a fluorescence microscope. Strong GFP signals were observed in the greater omenta and peritoneal linings of mice that received MV-GFP but not MV-αFR (Fig. 4B). GFP-positive cells were also present on medialstinal lymph nodes and spleens of MV-GFP–infected mice (data not shown). These GFP-positive cells were previously shown to be macrophages that concentrate in milky spots on the omentum, peritoneal lining, and marginal zone of the white pulp (30). Thus, the predominant non-target cells that were efficiently infected by MV-GFP are not permissive to infection by the fully retargeted MV-αFR. Other major organs (liver, kidneys, heart, and brain) were negative for GFP expression. However, it is also important to add that delivery of low levels of virus to these normal tissues, not detected using this method, cannot be excluded.

In vivo *antitumor activity of MV-αFR.* We first tested the oncolytic potential of MV-αFR *in vivo* via i.t. administration in a s.c. model of human ovarian cancer using FRα-positive SKOV3ip.1 cells. The FRα-targeted virus induced significant inhibition of tumor growth compared with the saline-treated controls (Fig. 5A). Repeated measures ANOVA showed a statistically significant difference in tumor growth between the MV-αFR–treated and control groups ($P_{treatment} < 0.0001$,

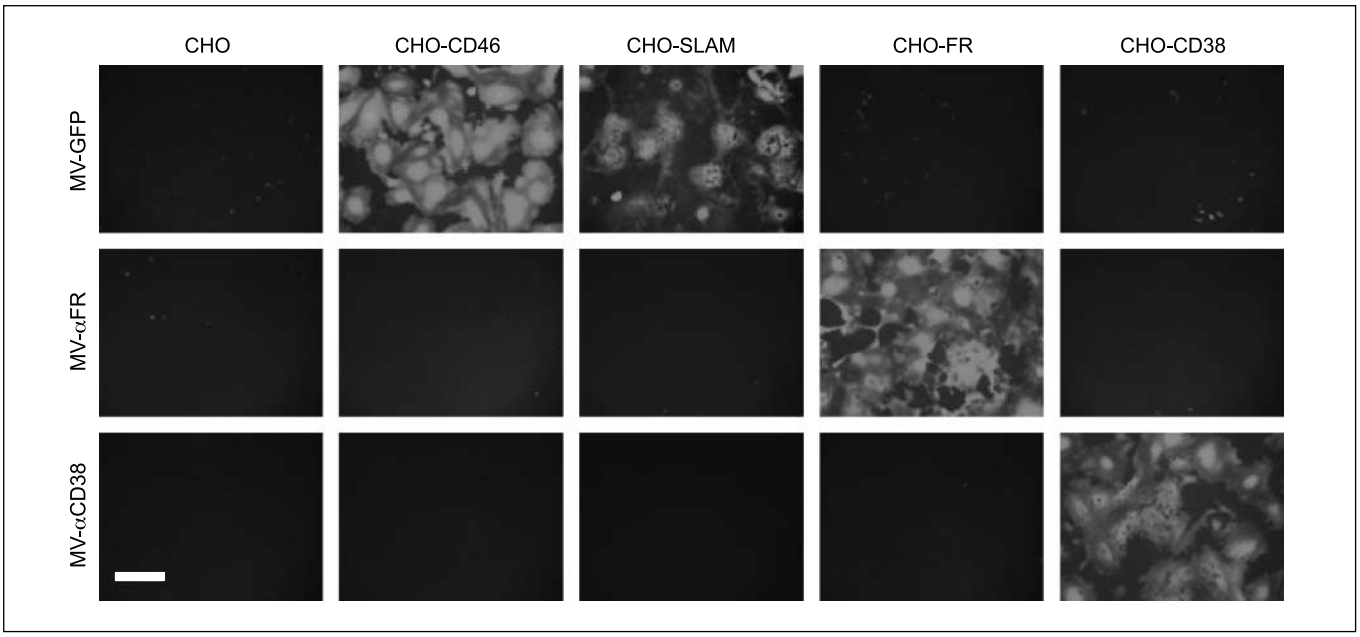

Fig. 2. Specificity of FRα-targeted measles virus. The CHO transfectants expressing CD46, SLAM, FRα, and CD38 receptors were infected with respective viruses at a multiplicity of infection of 0.5, and photographs were taken 2 days later. Bar, 0.5 mm.

$P_{time} < 0.0001$, $P_{interaction} = 0.034$). Therapeutic potency of the αFR-targeted measles virus was comparable with parental virus killed ovarian cancer cells through CD46 (24). From day 30, some mice in control group had to be euthanized due to tumor burden. In contrast, complete regression of tumors occurred in 5 of 10 and 3 of 10 mice treated with MV-αFR and MV-GFP, respectively.

We next tested the viruses in an i.p. model of disseminated ovarian cancer using SKOV3ip.1 cells stably expressing firefly luciferase and secreted βhCG. I.p. tumor growth was monitored noninvasively using bioluminescence imaging and analysis of plasma βhCG levels. At 4 days after cell implantation, growing i.p. tumors were detected using bioluminescence imaging and found to localize mainly at the greater omentum (Fig. 5B). Mice were treated with the viruses from day 6, every other day for a total of six doses (total dose = 6×10^6 $TCID_{50}$). Bioluminescence images revealed that there was significant inhibition of tumor growth in the MV-αFR–treated group compared with the saline control group (Fig. 5B). Differences in tumor burden were quantitated by measuring whole abdominal photon counts and plasma levels of tumor-secreted βhCG (Fig. 5C and D). Two-way repeated measures ANOVA (from day 4 or 6 to day 36) indicated that MV-αFR significantly reduced tumor growth compared with the saline-treated control (photon counts: $P_{treatment} = 0.0006$, $P_{time} = 0.0001$, $P_{interaction} = 0.0068$ and βhCG: $P_{treatment} < 0.0001$, $P_{time} < 0.0001$, $P_{interaction} < 0.0001$). There was 10-fold less tumor burden in the MV-αFR–treated group compared with control group on day 36 (photon counts: $P = 0.009$ and βhCG: $P < 0.0001$, unpaired t test). Survival of mice treated with MV-αFR was superior to that of the control group ($P < 0.0001$, log-rank test; Fig. 5E). Five of 10 MV-αFR–treated mice and 3 of 10 MV-GFP–treated mice were alive at the end of the experiment (day 90). All of these mice were euthanized, and gross examination of mice revealed no residual i.p. tumors, although some of them still had a s.c. injection site tumor.

Discussion

Here, we have generated an oncolytic measles virus with tight specificity for FRα receptor and have shown the virus to be therapeutically active in human ovarian cancer xenograft models. FRα is a very attractive cancer target. It is expressed in 70% and 40%, respectively, of ovarian and breast cancer cases seen in our cancer clinic (Supplementary Fig. S1B).[6] In contrast, its expression on normal tissues is restricted to apical surfaces of polarized epithelial where it has limited contact with circulating cytotoxic drugs (14, 15, 17). This study adds a new class of anticancer agent, an oncolytic virus, to the repertoire of FR-targeted experimental therapeutics (31) that already include monoclonal antibodies (21, 32, 33), bispecific antibody-targeted T cells (22, 23, 34, 35), and DNA vaccines (36). Because the cytotoxic agent typically reaches only a small percentage of cells in the tumor, live viruses are very attractive as anticancer agents as they can replicate and potentially spread from the initial infected cell to surrounding cell layers. An additional dimension to measles virotherapy is its mechanism of tumor cell killing through induction of extensive intercellular fusion between the infected cell and neighboring uninfected cells and significantly increasing the bystander killing index of this agent (8, 24).

Use of replication-competent viruses for cytoreductive cancer therapy (virotherapy) is not new. Various human and animal RNA viruses have been injected into cancer patients in the 1950s to 1970s, and results have both been intriguing and promising (37). Tumor selectivity is typically conferred by

[6] L.C. Hartmann, unpublished data.

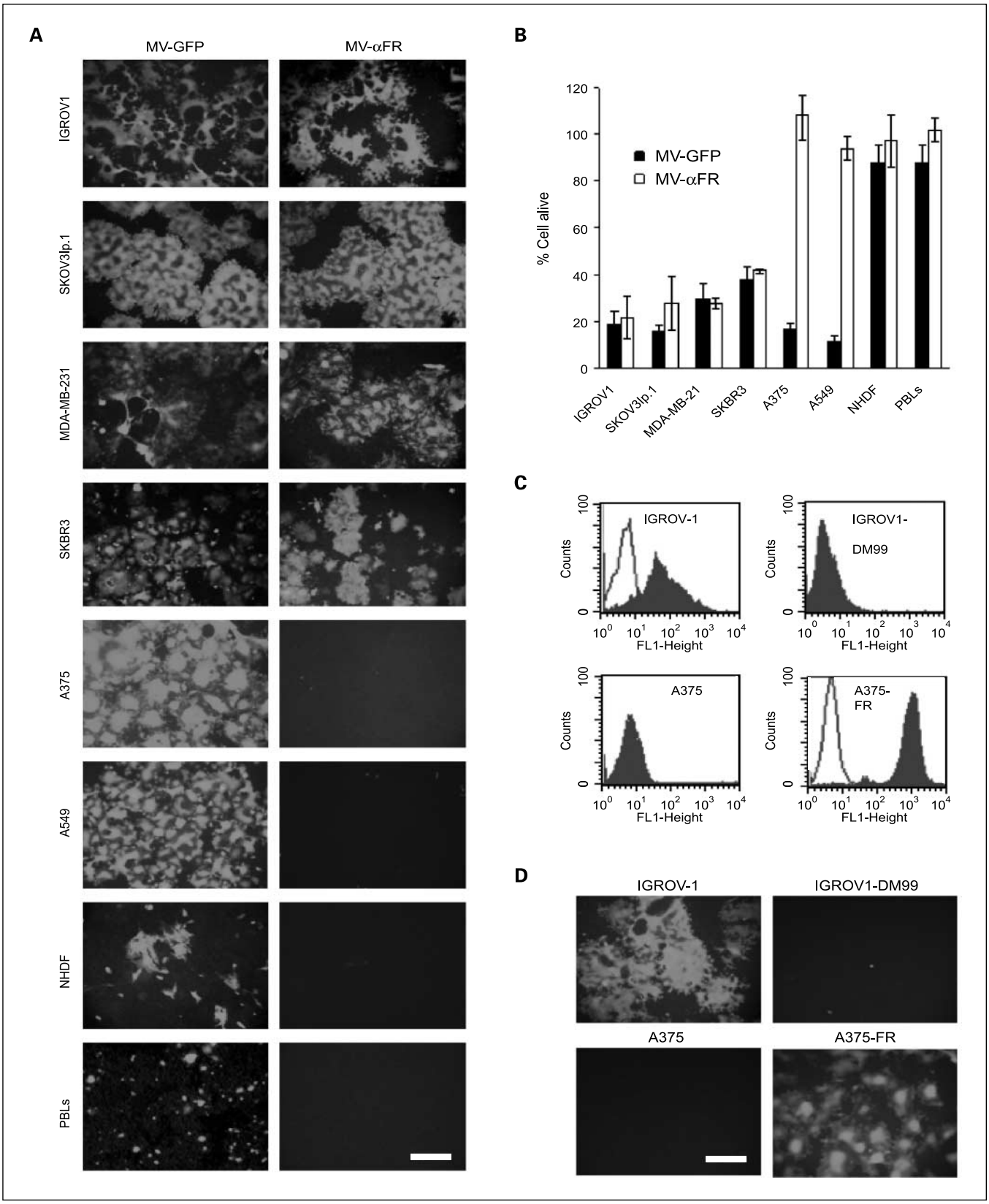

Fig. 3. *In vitro* tumor selective killing by FRα-targeted measles virus. Human tumor cell lines and normal cells were infected with the respective viruses at a multiplicity of infection of 0.5. *A,* photographed 2 days after infection. *B,* numbers of viable cells were counted by trypan blue exclusion at 3 days after infection. Columns, means from three replicates; bars, SD. Solid columns, MV-GFP; open columns, MV-αFR. *C,* flow cytometry data on FR expression (*filled histogram*) in IGROV-1 and A375-FR, IGROV-DM99, and A375 compared with isotype control (*empty histogram*). *D,* cells were infected with MV-αFR (multiplicity of infection = 0.5) and photographed 2 days after infection. Bar, 0.5 and 0.2 mm for peripheral blood lymphocytes.

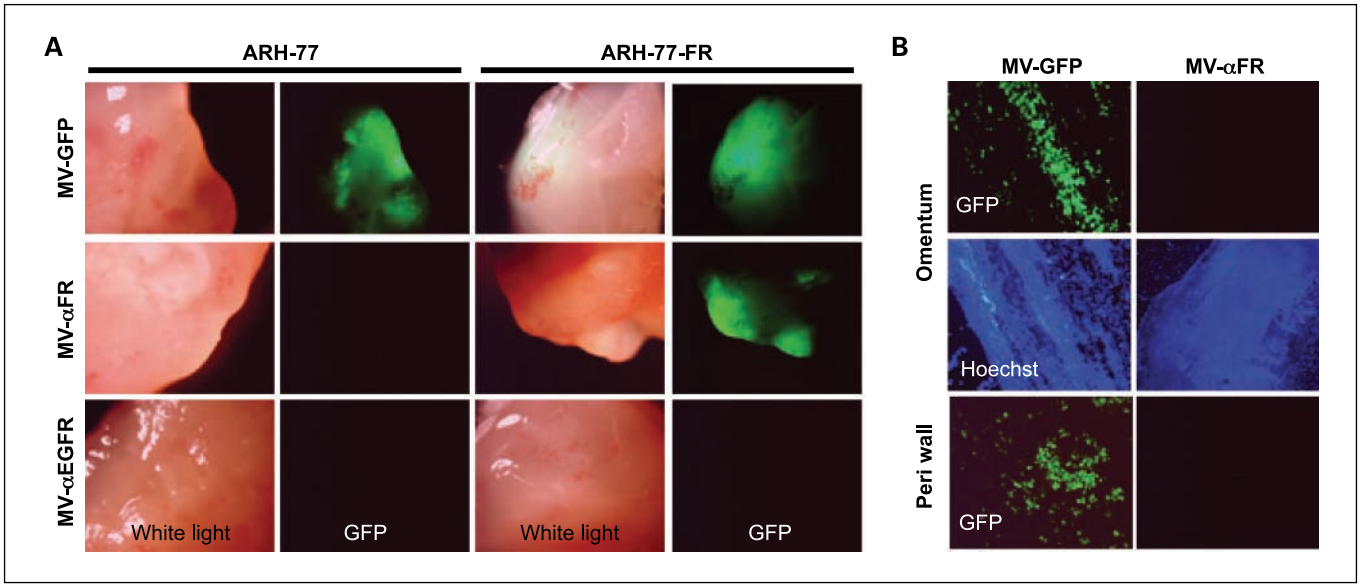

Fig. 4. *In vivo* specificity of MV-αFR. *A,* severe combined immunodeficient mice bearing s.c. ARH-77 or ARH-77-FR xenografts were injected twice i.v. via the tail vein (n = 3 per group) with 2×10^6 $TCID_{50}$ viruses. Tumors were harvested 4 days later and examined under white light or blue light (GFP fluorescence). Photographs of representative tumors. *B*, omentum and peritoneal linings from Ifnar-CD46Ge transgenic mice that received 2×10^6 $TCID_{50}$ MV-GFP or MV-αFR i.p. Tissues were harvested 2 days later, stained with Hoechst 33342, and examined under blue light for GFP fluorescence.

innate cellular antiviral defenses that protect normal tissues from unwanted damage. These protective mechanisms are in general defective in tumor cells, making them permissive to viral infections (38). Recent progresses in molecular engineering and virus rescue systems have enabled us to control tumor selectivity in new generations of "designer" tumor-selective viruses; these strategies either target defects in the intracellular genetic pathways or receptor usage (39, 40). Here, we have ablated the native tropisms of measles virus and redirected virus attachment, entry, and cytopathic effects to the tumor antigen via a scFv. Because measles is a negative-strand RNA virus with an estimated genomic mutation rate of 1.43 per replication (41), there is a possibility that the mutations introduced into the H protein are not stable, and reversion mutants that regain CD46 or SLAM usage might arise after serial passages of the virus in culture. Efforts have been made to minimize that possibility during the design of those mutations (42), and these fully retargeted measles viruses have been remarkably stable in their receptor usage after multiple serial passages in receptor-positive and receptor-negative cells (12, 43).

Accessibility of the tumor antigen to virus in the circulation is paramount to ensure efficient virus delivery to and infection of the tumor cells. Because ovarian cancer is localized mainly in the peritoneal cavity, i.p. administration of the virus puts it into direct contact with the cancer cells, bypassing the need for vascular delivery. We also showed here that after systemic administration into tumor-bearing mice, the MV-αFR virus was able to attach to and infect FR-positive tumor xenografts. Its access to the tumor antigens was probably facilitated by the leaky tumor vasculature (44). A strategy to improve vector localization is to target the virus to antigens on tumor neovessels (45). Identification of unique antigens on tumor neovessels, the availability of ligands for these targets, and the robustness of the vector retargeting technology are pertinent to the success of this endeavor. The measles retargeting technology is highly flexible; it can accommodate additional large polypeptides, such as scFvs, to mediate efficient virus entry and achieve titers that are comparable with that of the parental unmodified virus. Clearly, not all ligands displayed on measles virus will be functional, but this technology is a significant improvement compared with retroviral display (46). In fact, the scFv used in this study was first generated and displayed on the murine leukemia retrovirus. Vector attachment to FRα was redirected, but gene delivery was not achieved (47). Display of scFvs on adenoviruses remains challenging as the folding of scFv occurs in the endoplasmic reticulum, whereas adenoviral assembly does not (48, 49).

Interaction of wild-type measles virus with SLAM causes a profound but transient immunosuppression in infected individuals (11, 50, 51). Attenuated measles viruses, although less immunosuppressive, also use CD46 that is ubiquitously expressed at low levels on all nucleated cells. We hypothesize that ablation of CD46 and SLAM interactions should enhance the safety profiles, especially at the higher dose levels used in cancer therapy, of these fully retargeted viruses. Our preliminary studies using mixed lymphocyte reaction assays indicated that these fully retargeted viruses with ablated tropisms for CD46/SLAM do not inhibit lymphocyte proliferation.[7] In this study, we have also attempted to address potential decrease in toxicity by using measles susceptible genetically modified mice that express human CD46 with the same tissue specificity (29). Macrophages that were the predominant cell type infected by the parental virus were not infected by MV-αFR. Ablation of CD46 tropism could also minimize vector wastage to non-target cells, potentially enhance virus availability to the tumor cells, and achieve a more favorable therapeutic outcome. Because these transgenic mice do not also express the human FRα, it is difficult to evaluate potential toxicity of the virus and will require testing in non-human primates before initiation of a clinical study. In addition,

[7] T. Nakamura, unpublished data.

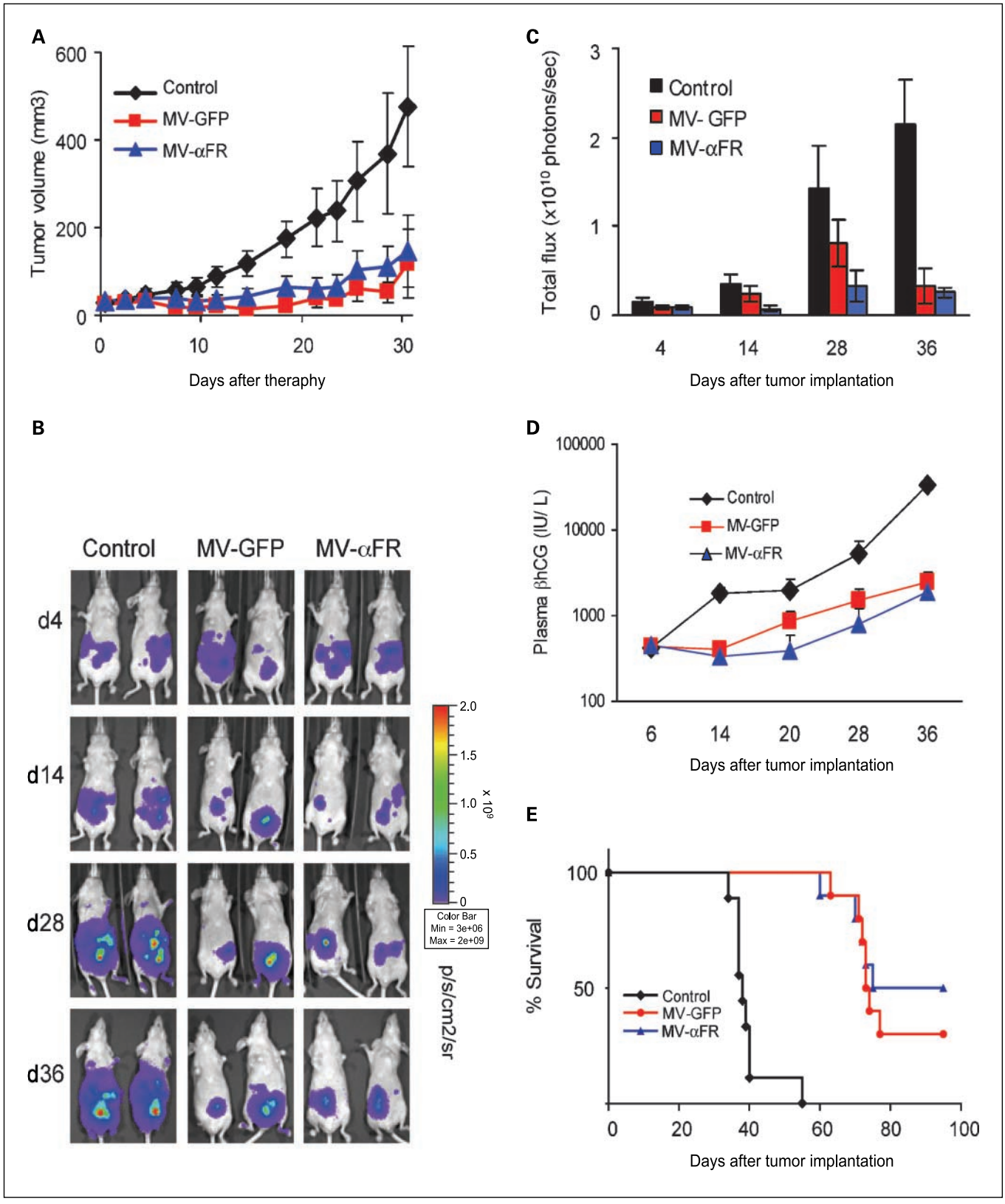

Fig. 5. *In vivo* antitumor activity of MV-αFR. *A,* tumor growth curves of s.c. SKOV3ip.1 xenografts treated by i.t. virus administration. Mice received a total of four doses of each virus at 5×10^5 $TCID_{50}$ per dose ($n = 10$ mice per virus treated group) or saline (control, $n = 9$). Tumor volumes were calculated using the formula: $0.5 \times \text{length} \times \text{width}^2$. *B* to *E,* i.p. model of ovarian cancer. SKOV3ip.1-Fluc-βhCG cells were injected i.p., and 6 days later, mice received a total of six doses of 1×10^6 $TCID_{50}$ of MV-GFP or MV-αFR i.p. ($n = 9$ for saline control and $n = 10$ for treatment groups). *B,* representative images of mice from bioluminescent imaging. Quantitation of tumor burden from (*C*) bioluminescent imaging or by (*D*) measuring plasma βhCG levels. Photon counts were calculated from the imaging data using the IVIS Living Image software. *E,* Kaplan-Meier survival curves of mice in the i.p. model. Points, means; bars, SE.

the true toxicity profiles of these retargeted viruses would be revealed in a careful dose escalation clinical trial.

Clearly, one of the major issues with use of measles virotherapy is the presence of preexisting neutralizing antibodies that could inhibit virus infectivity. Currently, we have no good methods to deplete these neutralizing antibodies, although it is an area under active investigation in our laboratories. Because ovarian cancer cells are localized mainly in the peritoneal cavity, intracavity administration of the virus is a feasible option to bypass antibodies in the circulation. This is the basis for the current trial to use i.p. instead of intravascular delivery. Virus inactivation is a balance between neutralization kinetics and virus numbers; it might be possible for some of the input virus to reach the target site before all viruses are neutralized. Indeed, sera with low titers of anti-measles antibodies are less effective at neutralizing a given titer of input virus.[8]

[8] K.-W. Peng, unpublished data.

In conclusion, MV-αFR is a new generation of oncolytic measles virus with tight tumor specificity and promising antitumor activity. The use of FR as a target also allows for image-guided therapy by combining folate imaging with measles virotherapy (52). Tc-99 m chelated EC20, a peptide derivative of folate (53), is being investigated clinically as a noninvasive radiodiagnostic imaging agent for the detection and monitoring of FR-positive cancers. It is envisaged that the use of a folate-imaging agent could greatly facilitate noninvasive monitoring of FR-positive tumors before and during the course of FRα-targeted measles virotherapy.

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