Fluorescence-Assisted Cytological Testing (FACT): *Ex Vivo* Viral Method for Enhancing Detection of Rare Cancer Cells in Body Fluids

Prasad S Adusumilli,[1] Sepideh Gholami,[1] Yun Shin Chun,[1] Michael Mullerad,[1] Mei Ki Chan,[1] Zhenkun Yu,[1] Leah Ben-Porat,[2] Valerie W Rusch,[1] and Yuman Fong[1]

[1]Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York, United States of America; and [2]Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, New York, United States of America

Cytological analysis of body fluids is currently used for detecting cancer. The objective of this study was to determine if the herpes virus carrying an enhanced green fluorescent protein (EGFP) could detect rare cancer cells in body fluids against millions of normal cells. Human cancer cells suspended with normal murine cells were infected with NV1066 at a multiplicity of infection (MOI) of 0.5 and 1.0 for 18 h. Fluorescent microscopy and flow cytometry were used for EGFP detection of cancer cells. EGFP-expressing cells were confirmed as cancer cells with specific markers by immunohistochemistry staining. Limits of detection of cancer cells in body fluid were measured by serial dilutions. Applicability of technique was confirmed with samples from patients with malignant pleural effusions. NV1066 expressed EGFP in 111 human cancer cell lines detected by fluorescent microscopy at an MOI of 0.5. NV1066 selectively infected cancer cells and spared normal cells as confirmed by immunohistochemistry. Sensitivity of detecting fluorescent green cells was 92% (confidence interval (CI) 83% to 97%) at a ratio of 1 cancer cell to 1 million normal cells. EGFP-positive cells were detected by fluorescent microscopy in patients' malignant pleural effusion samples. Our data show proof of the concept that NV1066-induced EGFP expression allows detection of a single cancer cell against a background of 1 million normal cells. This method was demonstrated to be a reliable screening tool for human cancer cells in a suspension of normal murine cells as well as clinical specimens of malignant pleural effusions.
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INTRODUCTION

Early detection of cancer before it has had a chance to metastasize remains the best strategy for reducing cancer mortality (1). Screening methods directed at early detection of cancers in individuals at high risk have been used with the aim of identifying cancers at a potentially curable stage (2–5). One routinely used method for screening high-risk patients involves microscopic examination of body fluids (for example, sputum and urine) for the presence of tumor cells (5,6). Such cytological tests are labor intensive and highly depend on the skill of the cytopathologist. The sensitivity of such sputum or urinary cytology studies is also governed by technical limitations of identifying the few cancer cells in the background of many normal cells. In this report, we present the technique of using recombinant herpes virus–expressing enhanced green fluorescent protein (EGFP) to detect rare cancer cells in body fluids.

Recent studies of oncolytic viral therapy have focused on genetically engineered viruses that are more specific in infecting cancer cells and thus less toxic to humans. One such promising candidate virus for human therapy is the herpes simplex virus (HSV). The HSV in the current study is a third-generation, genetically engineered multi-mutated HSV that has high specificity infecting tumor cells (7–10). A number of studies from our group and others have determined that these viruses are highly selective in infecting many tumor types, including lung, mesothelioma, bladder, head and neck, breast, esophageal, cervical, colorectal and gastric cancer, and they spare normal cells (9,11–15).

Currently, there is a lack of sensitive early-detection methods of rare cancer cells in body fluids. We therefore hypothesized that the tumor specificity of this class of viruses can be exploited for early detection of cancer cells in body fluids. Specifically, we examined the potential of enhancing the detection of rare cancer cells among millions of normal cells in body fluids by means of viral-induced cancer cell–specific EGFP expression.

***Address correspondence and reprint requests to** Yuman Fong, Murray F. Brennan Chair in Surgery, Department of Surgery, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065. Phone: 212-639-2016; Fax: 646-422-2358; E-mail: fongy@mskcc.org.*
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MATERIALS AND METHODS

Tumor Cell Infectivity Studies

A total of 111 human cancer cell lines, obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and other independent investigators from 16 different primary organs, were used to test for viral infectivity and EGFP expression. Cells were maintained in appropriate media and were incubated in a humidified incubator supplied with 5% CO_2 at 37°C. A total of 1×10^5 cells of each human cancer cell line was infected with NV1066 at a multiplicity of infection (MOI) (ratio of viral plaque-forming units [pfu] to cancer cells) of 0.5 or 1 *in vitro*. EGFP expression in cells was detected by using fluorescent microscopy at various time points after viral infection.

Cell Suspension for Detection Studies Using Fluorescent Microscopy and Flow Cytometry

For both experiments, 10 human lung (A549) and bladder (KU19-19) cancer cells were mixed with 1×10^6 normal cells from murine bronchoalveolar lavage or collected urine which was infected with NV1066 at an MOI of 0.5 or 1 for 18 h. In experiments involving fluorescent detection using microscopy, samples were directly mixed and incubated in chamber slides (Lab-Tek; Nalge Nunc, Rochester, NY). In experiments for flow cytometry detection, cells were mixed directly in flow cytometry polystyrene tubes. Following incubation, samples were centrifuged, supernatants were discarded, and the cell pellets were resuspended in 1 mL phosphate-buffered saline (PBS) for flow cytometric analysis.

Confirmation of EGFP-Expressing Cells as Cancer Cells

To confirm that green cells were indeed cancer cells, 1×10^5 cells from A549 (lung cancer) and NCI-H28 (mesothelioma) cell lines were spiked with normal lung cells harvested from mice without cancer. Noncancerous cells were obtained from organs from mice after euthanasia. Organs were minced, incubated with collagenase *in vitro*, filtered through nylon mesh and isolated by centrifugation. Cell suspensions were incubated with NV1066 (1×10^7 pfu) for 18 h. After that, the mixtures of cells were harvested and washed with PBS and bovine serum albumin. Cell aggregates were disrupted gently using a pipette, and the resulting single cell suspensions were incubated with R-phycoerythrin (R-PE)-conjugated mouse antihuman CD 51/61 monoclonal antibody, specific to both lung cancer and mesothelioma cells, for 30 min on ice. Samples containing antibody-stained cancer cells spiked with normal cells were analyzed by both fluorescent microscopy and FACScan flow cytometry. For microscopic detection, cells were differentiated from background artifacts on glass slides, if any, by staining live cells with Hoechst stain. Slides were initially observed under a bright-field and then examined for the presence of green cells; once a green cell was identified, the cell was confirmed to be a cancer cell by identifying the nucleus (stained blue when examined under a 4′,6-diamidino-2-phenylindole [DAPI] filter) and by identifying an R-PE–conjugated integrin surface antigen filter (stained red when examined under tetramethylrhodamine isothiocyanate).

Limits of Fluorescent Detection and Sensitivity Studies

To measure the limit of detection of tumor cells in body fluids, serial dilutions of cancer cells were used to spike normal cells. Lung cancer cells (0, 10, 100, 1,000, 10,000, or 100,000) were added to tubes that contained 10 million normal cells (from bronchoalveolar lavage from mice). In experiments involving fluorescent detection using microscopy, the samples were directly mixed and incubated in chamber slides to avoid any cell loss. In experiments involving flow cytometry, samples were mixed and incubated directly in flow cytometry polystyrene tubes. After gentle mixing of the samples, 1×10^7 pfu of NV1066 was added to all the samples except negative controls. After incubation, samples were centrifuged, the supernatant was discarded and the cell pellet was resuspended in 1 mL PBS for analysis. Each sample was analyzed in six replicates. For fluorescent microscopy, the slides were labeled randomly. Ten observers from three different laboratories from this academic institution who were blinded to the actual concentration of mixture of cancer cells and normal cells and the presence of green cells in each sample reviewed these slides.

Patient Pleural Fluid Samples

All studies were performed after consent under an institutional review board–approved protocol for tissue and fluid collection was obtained. Pleural fluid was collected from two patients with malignant pleural effusions and one patient without cancer but who was subjected to placement of a chest tube intraoperatively. Patients were selected randomly from the inpatient service at Memorial Sloan-Kettering Cancer Center. Specimens were obtained from the tube drainage and immediately transported on ice to the laboratory. For each patient, 10 separate aliquots were acquired (total samples with suspected cancer, n = 20; total controls, n = 10). The specimens were centrifuged at $129g \times 5$ min at 4°C and resuspended in Roswell Park Memorial Institute (RPMI) medium with 10% fetal calf serum, 100 μg/mL penicillin and 100 μg/mL streptomycin. Cells were counted using a hemocytometer, and 5×10^5 cells were aliquoted into polystyrene round-bottom tubes (BD Falcon, San Jose, CA, USA). The 30 experimental aliquots were incubated each with 2×10^5 pfu of NV1066. After an 8-h incubation, cells were washed with PBS and cytospun for 4 min at 200*g* onto silane-coated slides (Electron Microscopy Sciences, Hatfield, PA, USA). Slides were fixed with 1% paraformaldehyde at room temperature for 12 min, washed twice with PBS, and stained with acidophilic and basophilic stains from the Quick-Dip staining system (Mercedes Medical, Sarasota, FL, USA). Bright-field and fluorescence microscopy was performed using a Zeiss Axio Imag-

Table 1. Cancer cell lines that can be infected by NV1066 and express EGFP.

Primary organ of origin	Cell lines	Body fluid(s)
Gastrointestinal		Ascitic fluid, endoscopic biopsy or needle aspiration, nasogastric tube drainage, feces, rectal swab, needle aspiration cytology from lymph nodes
Esophagus	BE3, SKG-T4	
Stomach	OCUM, MKN-1, MKN-45, MKN-74	
Colorectal	CT26, HCT-8, HCT-116, HT-29, HT-29 MDR	
Hepatobiliary		Ascites, liver biopsy, needle aspiration cytology, biliary drainage
Hepatocellular	Hep G2, Hep 3B, PLC/PRF/5, SKHep1, SNU-182, SNU-354, SNU-368, SNU-387, SNU-398, SNU-423, SNU-449, SNU-475, SNU-739, SNU-761, SNU-878, SNU-886	
Cholangio	HUCCT-1, KMBC, SK-ChA-1, SNU-1079, SNU-1196, YoMi, mZ-ChA-1, TGBC-1, TGBC-2	
Pancreas	Pan02, AsPC-1, BxPC-3, hs766t, HTB147, Panc-1, MIAPaCa-2, SNU-478, SNU-869	Pancreatic fluid, ascites, endoscopic biopsy, needle aspiration cytology from lymph nodes
Lung	A549, H1299, 2030, H322, H522	Sputum, bronchial lavage, pleural effusion, mediastinal lymph node biopsy
Mesothelioma	JMN, VAMT, MSTO-211H, H-2373, H-2052, H-2452, H-28, Meso, Meso1A, Meso-9, Meso-10	Pleural effusion, ascites, mediastinal lymph node biopsy
Genitourinary	J82, RT4, T24, UMUC-3, SK UB	Urine, cystoscopic drainage, prostatic secretions, endoscopic biopsy
Bladder and prostate	DU-145, C4-2, CWR-22, CWR-22R, PC-3	
Breast	HCC1500, HCC1937, HCC 1954, M*CF*-7, M*CF*-7 MDR, MDA-MB-231, MDA-MB-435, MDA-MB-435LN, MDA-MB-435S, SkBr3, T47D	Breast ductal drainage, nipple discharge, needle aspiration cytology, bone marrow cytology
Head and neck	SCC VII, SCC15, SCC25, SCCQLL1, SCCQLL2, 686, 1586, 1986, 886, MSK922, MSK1493, LN1-LN7, MG1, MG11, MG14	Oral secretions, lymph node aspiration cytology, oral swab, throat swab
Thyroid	NPA-187, WRO 82-1, DRO81-1, DRO90-1, ARO, KAT-4C, KAT-18y	Needle aspiration cytolog
Uterine: cervix	HeLa	Pap smear

ing upright microscope (Carl Zeiss, Oberkochen, Germany) with a 100-W mercury arc lamp light source and Retiga EX CCD digital camera (Leeds Precision Instruments, Minneapolis, MN, USA). For detection of green fluorescence, selective excitation of EGFP was produced through a Chroma 41017 filter set (Chroma Imaging, Rockingham, VT, USA). Images were processed and analyzed with Velocity Imaging Software (Improvision, Lexington, MA). An attending pathologist blinded to the study performed conventional cytological assessment. The institutional animal care committee and IRB of Memorial Sloan-Kettering Cancer Center approved all protocols.

Herpes Simplex Virus

NV1066 is a novel, replication-competent, attenuated oncolytic herpes virus and has been described in our previous studies (16). It is a third-generation herpes viral construct with single copy deletion of $\alpha0$, $\alpha4$ and $\gamma34.5$ with results in decreased host neurovirulence and increased tumor specificity of the virus. It furthermore contains an EGFP cassette that causes infected cells to fluoresce green under the proper fluorescent microscopy.

Fluorescence Microscopy

A Zeiss LSM 510 confocal laser scanning microscope (Carl Zeiss, Oberkochen, Germany) and MetaMorph Imaging System (Universal Imaging Corporation, Downingtown, PA, USA) were used to visualize EGFP-expressing cancer cells. Imaging was performed in both bright-field and fluorescent modes. Live cells were identified by Hoechst staining, examined under a DAPI filter, and EGFP expression was identified after placement of both excitation and emission filters to detect EGFP.

Flow Cytometry

After incubation, samples were centrifuged, supernatants were discarded and the cell pellets were resuspended in 1 mL PBS for analysis. Infected cell suspensions were analyzed using a fluorescent channel 1 (FL-1) to detect green fluorescence via standard flow cytometry. Nonviable cells were identified by using 7-amino actinomycin D (7-AAD). Matched isotype controls were used in all flow cytometry panels. The mean intensity of the EGFP-positive cells was compared with the mean intensity of the EGFP-negative uninfected cells (samples without any NV1066) as a negative con-

trol and plotted. Standard flow cytometry was performed in accordance with guidelines outlined in the 1995 U.S.–Canadian consensus conference. Data acquisition analyses were performed on a FACScan flow cytometer (BD Biosciences, San Jose, CA, USA). CellQuest software (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) was used for data analysis. All flow cytometry experiments were repeated by two independent investigators to ensure reproducibility. Each experiment was repeated at least three times.

Statistical Analysis

Sensitivity and confidence intervals (CIs) for detecting green fluorescent cells were calculated using the Poisson distribution. A *P* value of <0.05 was considered statistically significant. Data are presented as mean ± standard deviation.

RESULTS

Detection

In vitro, NV1066 infected 111 types of cancer cell lines derived from 16 different primary organs (Table 1). NV1066-infected cancer cells expressed EGFP within 1–2 h. NV1066 infected cells and expressed EGFP at a low MOI of 0.5 in all cell lines. At a higher MOI of 1.0, the majority of the cancer cells in the sample were infected at an earlier time point and expressed strong EGFP fluorescence.

Fluorescence-assisted cytological testing (FACT) could easily identify rare green cells in a mixture of millions of normal cells. One cancer cell in a mixture of 1 million normal cells is difficult to identify under bright-field microscopy (Figure 1A). Under fluorescent microscopy, EGFP-positive NV1066-infected cells can be easily identified by means of green fluorescence (Figure 1B). Overlap of the fluorescent picture with bright-field identifies the cancer cell (Figure 1C) for further histological testing (upper panel shows experiment with lung cancer in brochoalveolar lavage; lower panel shows experiment with bladder cancer in urine).

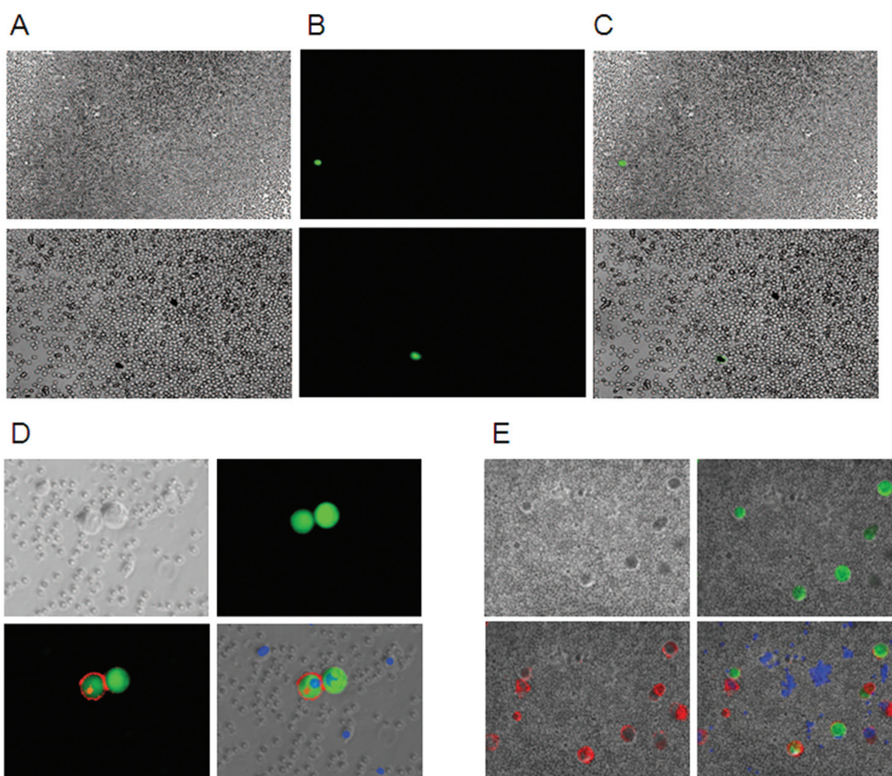

Figure 1. EGFP-positive cancer cells can be identified against a background of millions of normal cells. (A–C) A rare cancer cell in a mixture of millions of normal cells is difficult to identify under bright-field microscopy and is time-consuming (A). Under fluorescent microscopy, EGFP-positive NV1066-infected cancer cells can be easily identified by means of green fluorescence (B). Overlap of fluorescent picture with bright-field identifies the cancer cell (C) for further studies. (*Upper panel* shows mixture of lung cancer cells and normal cells in bronchoalveolar lavage, and *lower panel* demonstrates a mixture of bladder cancer and normal cells in urine.) A rare cancer cell among millions of cells can be detected and separated out for further studies by flow cytometry. (D) NV1066 selective infection of cancer cells among a mixture of millions of normal cells is confirmed by counterstaining with immunohistochemistry. Human mesothelioma cancer cells were mixed with normal pleural cells (*upper left panel*) and were incubated with NV1066 for 18 h. Examination under fluorescence microscope identified cancer cells by expression of strong green fluorescence (*upper right panel*). These cancer cells express integrin (CD 51/61) surface antigen. Incubation with R-PE–conjugated mouse antihuman CD51/61 monoclonal antibody confirmed that EGFP expression is selective to only cancer cells (identified by red fluorescence, *lower left panel*). Overlap of fluorescent pictures with bright-field identifies cancer cells among normal cells (*lower right panel*). Live cells among the cell clumps were identified by nuclear Hoechst staining (*blue*). (E) A similar experiment repeated with human lung cancer cells mixed with normal bronchoalveolar cells and incubated with NV1066 confirms similar results as described in (D).

To confirm NV1066 cancer cell selectivity, we combined green fluorescence detection with immunohistochemistry for cancer cells. Mixtures of malignant mesothelioma cells, NCI-H28 and normal pleural cells were incubated with NV1066 for 18 h. All green cells were confirmed to be cancer cells by positive counterstaining with R-PE–conjugated mouse antihuman CD51/61, expressed by mesothelioma cells (Figure 1D). A similar experiment was repeated with human lung cancer cells, and the results were reproduced (Figure 1E).

Limits of Detection and Sensitivity of FACT

A total mixture of 2,000,000 cancer and noncancerous cells was infected with NV1066 and sorted out by size and autofluorescence using a standard fluorescence-activated cell sorter (FACS) machine (detected in the FL-1 channel, Figure 2A). Cancer cells spiked at serial dilutions as low as 1 cancer cell per 1 million normal cells were easily identified by flow cytometry because of the strong green fluorescence (Figure 2B).

To identify the minimum number of cells required to obtain a sufficiently strong EGFP signal, lung cancer cells were mixed with normal cells from bronchoalveolar lavage in ratios from 1:10 to 1:1 million and incubated with NV1066 for 18 h. The mean fluorescence intensities of green fluorescence of EGFP-positive cells are plotted (Figure 2C). At a dilution of 1 cancer cell to 1 million normal cells, EGFP mean fluorescence intensity was >100. Because of this strong expression of EGFP, cancer cells in body fluids, even at a concentration of 1 cancer cell per 1 million normal cells, could easily be identified by fluorescent detection.

In the experiment where serial dilutions of cancer cells were spiked with millions of normal cells, 10 independent observers identified the presence or absence of green cells on a given slide. For each dilution, there were a total of 60 observations. Appropriate positive and negative controls were included in the analysis. Presence or absence of green cells was accurately identified (Table 2) with an overall sensitivity of 98% (CI 96% to 99%) by all observers, irrespective of their previous experience with microscopy or fluorescence detection. Even when the concentration of cancer cells was 1 per 1 million normal cells, the observers identified the presence of the green cell accurately with a sensitivity of 92% (CI 83% to 97%). In the absence of cancer cells in a negative control slide, 2 of the 10 observers identified a green cell, later identified as an artifact. This error was eliminated after the observers reexamined the greenness with a DAPI filter for lack of nuclear staining.

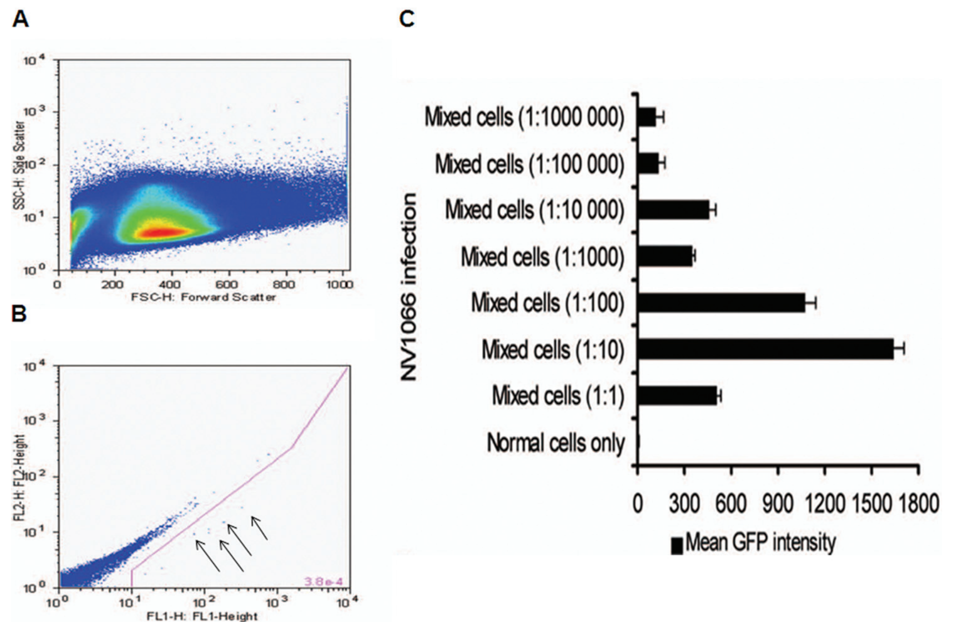

Figure 2. Detection of EGFP-positive cells by flow cytometry. (A) Two million cells were sorted out by their size and autofluorescence by flow cytometry. (B) Among the same cell population, cancer cells were identified by strong green fluorescence in the FL-1 channel. These rare cancer cells can be separated out for further histological studies by flow cytometric sorting. (C) Because of their strong expression of EGFP, NV1066-infected cancer cells in body fluids can be easily identified, even in a background of millions of cells or cell clumps. Lung cancer cells were mixed with normal cells from bronchoalveolar lavage in ratios from 1:10 to 1:1,000,000 and incubated with NV1066 for 18 h. Cancer cells mixed with NV1066 served as positive controls, and normal cells mixed with NV1066 served as negative controls. The mean fluorescence intensities of EGFP-expressing cells in each sample were plotted.

Clinical Application

To determine the clinical applicability of our technique, specimens obtained from patients with known cancer in the pleural fluid were compared with control specimens from patients without cancer from a chest tube placed for mechanical reasons. A small dose of NV1066 (2×10^5 pfu) detected lung cancer cells in all specimens tested from patients with malignant pleural effusions. All slides from patients with malignant pleural effusions (n = 20) were correctly identified by the presence of green fluorescence. No fluo-

Table 2. Identification of presence or absence of green cells.

Ratio of cancer cells to noncancerous cells	Slides observed (n)	Postive diagnosis for cancer (n)	Negative diagnosis for cancer (n)	Correct observations (% (CI))
0	60	2	58	97 (89–99)
1:10	60	60	0	100
1:100	60	60	0	100
1:1,000	60	60	0	100
1:10,000	60	60	0	100
1:100,000	60	57	3	95 (87–99)
1:1,000,000	60	55	5	92 (83–97)
1:10–1:1,000,000	360	352	8	98 (96–99)

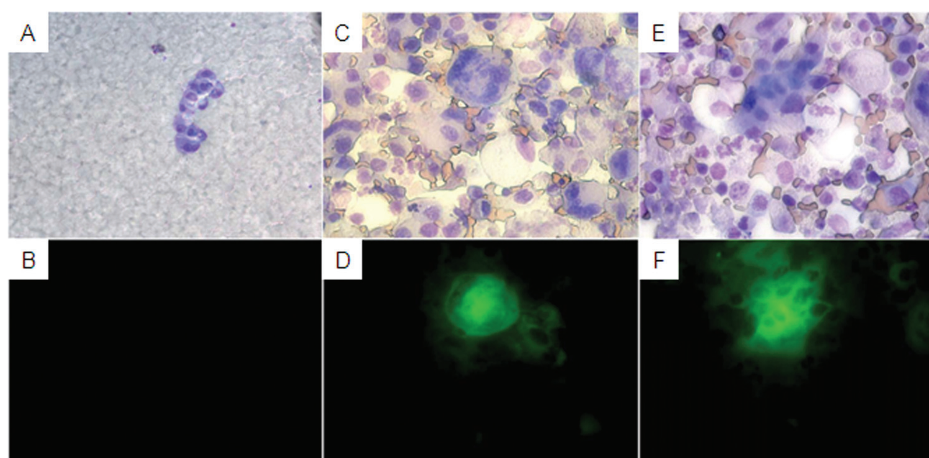

Figure 3. Detection of NV1066-infected cancer cells using EGFP fluorescence in human samples (magnification 30×). (A) Cytological examination showed benign mesothelial cells, as read by an attending pathologist. (B) These benign cells did not fluoresce when viewed under the green fluorescent protein (EGFP) filter. (C, E) Cytological examination showed malignant cells in samples from patients with non–small cell lung cancer. (D, F) Malignant cells expressed green fluorescence under the enhanced EGFP filter.

rescence was detected in any control samples (n = 10). Representative microscope slides are shown in Figure 3. None of the cellular elements, such as benign mesothelial cells (Figure 3A), showed fluorescence. Specimens with non–small cell lung cancer clearly expressed EGFP (Figure 3C–F).

DISCUSSION

One screening technique that is already in use is the examination of sputum, urine or cervical cytology in high-risk patients to look for cancer cells. These tests are attractive, since they are easily performed and pose little risk to the patient. However, examination of these specimens by current cytological techniques is labor intensive and low in sensitivity. For example, sputum analysis is widely used to screen the high-risk population for lung cancer. The U.S. National Cancer Institute early lung cancer study gave sputum cytology a sensitivity of 23% and 10% for prevalence and incidence, respectively (6). Even for conventional routinely used cervical smears, the sensitivity was reported by the Agency for Health Care Policy Research to be only 51% (17).

Given these limitations of standard cytopathological tests, several investigators have recently proposed automated techniques for detection of cancer cells (18–21). These techniques exploit the differences in cellular autofluorescence and cell size between malignant and normal cells (Figure 2A). Current methods of flow cytometric detection of malignant cells in body fluids, however, have been limited by the lack of uniform biomarker expression. Other methods including a magnetic bead–based separation technology propose a similar issue. With this method, cells are positively selected by means of antibodies to magnetic beads targeting epithelial markers or tumor-specific antigens. One general limitation for the enrichment method is that there is no "universal" marker, and therefore it cannot be applied to all tumor types. However, FACT technology exploits the tropism of a genetically modified HSV and can target a range of cancer cells.

Our current method of detecting malignant cells using fluorescent-assisted cytological testing offers several advantages. EGFP expressed after NV1066 infection is a stable protein with several-fold higher fluorescence than cellular autofluorescence and does not require additional substrates or cofactors for its expression. Furthermore, the fluorescence intensity of enhanced EGFP is pH insensitive (22), as shown by our experiments performed in bronchoalveolar lavage and urine. In addition, the combination of excitation and emission wavelengths for EGFP is specific and thus eliminates autofluorescence interference. Moreover, inflammatory cells often interfere with the diagnosis of cancer because of cell size, texture and fluorescence (18). Because these inflammatory cells have different excitation-emission wavelengths than EGFP (EGFP 475/509 nmol/L, tryptophan 290/330 nmol/L, NAD(P)H 350/450 nmol/L, FAD 450/530 nmol/L), a misdiagnosis by labeling inflammatory cells as cancer cells is avoided by FACT (18). In addition, in our current technique, clumps or sheets of cells do not cause a problem as in other methods (non-uniform staining by biomarker detection chemicals and variation in fluorescence). In fact, viruses spread better in clumps or confluent cells. Our results support that cancer cells spiked at serial dilutions with as low as 1 cancer cell per 1 million normal cells can be easily identified by flow cytometry because of the strong green fluorescence. Our data suggest that this FACT technique can easily identify and sort out rare cancer cells. FACT can be implemented with currently available equipment and personnel in most hospitals around the world at reasonable costs. The specimens that screen positive by FACT can be separated out by flow cytometric sorting and sent to a pathologist to further characterize the exact nature of malignancy. After fluorescence microscopic examination, the results can be confirmed by routine histological methods or the fluorescent cells can be sorted by flow cytometry for further characterization.

We acknowledge that using a mixture of human cancer cells and normal murine cells is a limitation to our study. However, we also included clinical samples in our study to show that the concept of FACT is applicable to human

cells. It would not have been a feasible experimental design to use human normal cells for the background, given that we performed the study in multiple cell lines. We further acknowledge that our sample size is small and plan to expand our study with a larger number of clinical samples in future experiments. Lastly, for certain body fluids, such as urine, the physiological pH varies depending on dietary intake and other factors. Therefore, the degree that these variables, such as pH and temperature, affect FACT still needs to be addressed in future specific studies for bladder cancer. This is a proof-of-concept study, and we plan to perform future studies to examine the clinical utility of specific cancers and clinical scenarios.

In conclusion, we present a novel, sensitive and accurate technique to label tumor cells with green fluorescence by infecting them with a recombinant engineered herpes virus. This method was demonstrated to be feasible, operator-independent and applicable to a range of cancers *in vitro* as well as shown to have potential application *in vivo*. Our current work presents an initial proof of concept, and the range of tumor tropism requires more testing in human clinical specimens, such as bronchial or peritoneal washings.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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