Replication-restricted vaccinia as a cytokine gene therapy vector in cancer: Persistent transgene expression despite antibody generation

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Background: As antitumoral immunity requires the generation of local immunity directed against tissue proteins, we attempted to recreate within tumors the same environment found within tissues affected by autoimmune diseases (i.e., prolonged cytokine expression). Vaccinia virus (VV) has not been widely used as a cytokine gene therapy vector because of presumed high immunogenicity that would likely make repeated injections impossible; therefore, we modified it by inserting the cytokine gene into the thymidine kinase region, rendering it replication-restricted. The cytokine chosen was human interleukin-2 (IL-2), a molecule with powerful antitumoral effects.

Methods: Six patients with the treatment-resistant tumor malignant mesothelioma received intratumoral (i.t.) VV-IL-2 therapy for 12 weeks by injection of 10⁷ plaque-forming units of VV-IL-2 per dose. Serial tumor biopsies, sputum, urine, and blood samples were tested for VV-IL-2 mRNA expression; VV culture and T-cell infiltrates were evaluated by immunohistochemistry. Patients and contacts of patients were monitored for changes in VV immunoglobulin G (IgG) levels and clinical evidence of VV infection.

Results: VV-IL-2 was not excreted and was only cultured in one patient from tumor biopsies. A T-cell infiltrate was detected in 50% of tumor biopsies. VV-IL-2 mRNA expression was highest on days 1–3 postinjection and was detected for up to 3 weeks after each injection even though VV IgG levels rose in all patients. No significant toxicities, infection of patient contacts, or tumor regressions were observed.

Conclusions: I.t. VV-IL-2 administration is safe, is associated with minimal toxicity, and results in i.t. expression of VV-IL-2 for up to 3 weeks postinjection regardless of the level of anti-VV IgG titers generated. This suggests that VV may be a good vector for repeated cytokine gene therapy of solid human cancer. **Cancer Gene Therapy (2000) 7, 663–670**

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Most of the identified human tumor T-cell antigens (Ags) are self Ags,¹ and it is believed that the type of immune response required to destroy an established tumor parallels that which is found in destructive, autoimmune diseases. This is encouraging, as autoimmune processes can effectively destroy human tissue, implying that if we can recreate the same conditions in cancer tissue, a prolonged immune attack may occur. Two conditions must be met for tumors to undergo immune-

mediated destruction. First, immunological events must occur *within* the tumor (i.e., the tumor must become infiltrated with activated T lymphocytes producing cytokines that augment host antitumoral activities).² Second, this process must occur over a relatively prolonged period of time.³

To generate high concentrations of cytokines within a tumor environment over prolonged periods, two main therapeutic options exist. First, cytokines can be constantly infused into tumor tissue.⁴ This process is limited by the technical difficulties of inserting and maintaining intratumoral (i.t.) catheters, by problems related to recombinant cytokine distribution within the tumor, and by unexpected side effects. Second, cytokines can be induced in the tumor by cytokine gene transfer (i.e., by injecting the relevant cytokine gene in such a way that

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the tumor cells themselves produce cytokines).⁵ This approach has the theoretical attraction of inducing the prolonged expression of high concentrations of cytokine within the tumor milieu for prolonged periods after each injection.

Most clinical trials of human cancer gene therapy have used retrovirus or adenovirus as vectors.⁵⁻⁷ Vaccinia virus (VV) has not been used previously as a vector for cytokines in patients with malignancy. This is largely related to the presumed theoretical disadvantage of its inherent immunogenicity and the likely induction of high levels of anti-VV antibodies (Abs), which might be expected to limit the effectiveness of repeated doses of VV-cytokine constructs. We chose to use VV in this study because its virology and safety have been well-described, having been used in millions of individuals worldwide in the control of smallpox. We also reasoned that insertion of the interleukin-2 (IL-2) gene into the thymidine kinase (TK) region of the VV would render it replication-restricted. This might allow more prolonged expression within tumor cells, where TK is produced at higher levels than in normal tissues. Substantial animal data exist supporting the notion that VV-cytokine constructs can have profound effects on host immune responsiveness.⁸⁻¹¹

We chose to study VV-IL-2 in malignant mesothelioma (MM), an aggressive tumor that arises from serosal surfaces such as pleura and peritoneum.¹² MM is increasing in incidence throughout the world, largely due to widespread exposure to asbestos; it is uniformly fatal and is resistant to all standard forms of therapy.¹³ There is an accumulating body of data that supports the concept that MM may be susceptible to immunotherapeutic approaches.^{14,15} The majority of these data are based on the murine model of MM, which almost exactly mimics its human counterpart in terms of pathology, biology, immunology, and clinical behavior.¹⁶ There are also limited data from humans with MM supporting the concept that under some circumstances this disease may be susceptible to immunological approaches, such as the relationship between tumor-infiltrating lymphocyte proportions and prognosis,¹⁷ partial responsiveness to the systemic immunomodulatory agent interferon- α ,¹⁸ rare transient spontaneous regressions associated with lymphocytic infiltration (our unpublished observations), and our recently demonstrated association between MM regression and mononuclear cell infiltration in patients undergoing continuous intralesional infusion of recombinant granulocyte-macrophage colony-stimulating factor.⁴

IL-2 had powerful anti-MM effects in our animal studies using cytokine gene transfer approaches in MM;¹⁹ on that basis, this cytokine was chosen for our initial human clinical study.

The pilot study reported here was established to determine the safety of the vector, particularly in terms of toxicity for the patient and possible transmission to contacts, the level and duration of gene expression after vector injection into the tumor, the biological effects induced by this approach within the tumor, and antitumor activity. The results demonstrate that VV-IL-2,

when administered i.t. to a maximum dose of 10^7 plaque-forming units (PFU) per patient per week, is without significant toxicity, is not transmitted, and, importantly, induces VV-IL-2 mRNA expression for up to 3 weeks after each injection, regardless of the level of systemic anti-VV immunity, inducing modest biological effects within the region of injection.

MATERIALS AND METHODS

Patients

Eligible patients were those with confirmed histological or cytological diagnosis of MM, measurable disease in the form of a subcutaneous (s.c.) nodule protruding through the chest wall, life expectancy of ≥ 12 weeks at enrollment, symptoms attributable to MM, and normal hematological and biochemical parameters.

Protocol

Written informed consent was obtained from each patient before commencement of the study. The study was approved by the Human Rights Committee of the University of Western Australia, the Clinical Drug Trial Committee of Sir Charles Gairdner Hospital, and the Gene Therapy Committee of the National Health and Medical Research Council of Australia.

All staff involved in the study and close contacts of the patients received vaccinia vaccination, supplied by Commonwealth Serum Laboratories (Melbourne, Australia). The vaccine was delivered intradermally to the deltoid region by a standard multiple pressure technique using a 21-gauge needle. All individuals showed evidence of vaccine replication at the inoculation site.

Six patients were enrolled. Injection of VV-IL-2 was performed by insertion of a 21-gauge needle ~ 5 cm into the center of the s.c. nodule. A single needle track was made, and the injection of 1 mL volume was performed in a volume of ~ 5 mL of tumor by maneuvering the needle slightly within the tumor to increase the volume of tumor exposed to the virus. Because of an early theoretical risk of disseminated VV infection, patient 1 received a pretreatment VV revaccination. This patient then had an initial dose of VV-IL-2 of 10^5 PFU, with dose escalation over the next two cycles to 10^7 PFU. Because of low VV-IL-2 yields from the injection site of this first patient, the remaining patients did not receive VV vaccination before commencement of therapy.

Patients 1 through 4 had three weekly VV-IL-2 injections. As polymerase chain reaction (PCR) analysis of VV-IL-2 expression showed that transgene expression fell after 1 week, patients 5 and 6 received weekly i.t. injections of 10^7 PFU. In patients 1 and 2, tumor biopsies and other specimens, as listed below, were obtained before each treatment and subsequently on days 3 and 8 of each cycle. Tumor biopsies and other specimens, as listed below, were obtained from patients 3 and 4 before each treatment and on days 1 and 7 of each cycle. In patients 5 and 6, tumor biopsies and other samples were obtained before each treatment and on day 1 of each cycle. At least three tumor biopsies were taken in all patients from the same area of tumor tissue that had been injected with VV-IL-2 using a 14-gauge Tru-cut biopsy needle (Baxter, Deerfield, Ill) on sampling days as listed above. Chest x-rays and computed tomography (CT) scans were performed immediately before the beginning of treatment and at the conclusion of the treatment in each patient. In addition, chest radiographs were performed at three to four weekly intervals during the study.

Direct measurement of the s.c. nodule of MM being injected was performed weekly.

Preparation of VV-IL-2

A recombinant VV expressing the human IL-2 gene was constructed by isolating a 1-kb *Xho*I from pcD-IL-2 (K. I. Arai, DNAX Research Institute, Palo Alto, Calif). A 500-bp human IL-2 cDNA was removed using an *Rsa*I restriction enzyme and was inserted into plasmid pBCB07. This plasmid was introduced into the TK gene in the J region of the NYCBOH strain of VV. TK-negative recombinant viruses were then selected for IL-2 expression. (A 143B fibroblast cell line was used to produce the initial VV-IL-2.) The recombinant virus expressing IL-2 was plaque-purified and grown in fibroblasts harvested from specific pathogen-free chicken embryos, under conditions appropriate to the Therapeutic Goods Act. The recombinant virus vaccine has been reviewed by the Therapeutic Goods Administration Laboratories.

To test the production of IL-2 *in vitro*, 10⁶ cells of two human mesothelioma cell lines (JU77 and NO36) were cultured and infected with VV-IL-2 at a multiplicity of infection (MOI) of 0.5 for 24 and 72 hours, respectively; the supernatant collected and assayed for IL-2 by enzyme-linked immunosorbent assay (Genzyme, Cambridge, Mass).

Evaluation of toxicity

Toxicity was assessed by the development of fever, malaise, nausea, vomiting, persistent tumor pain (not attributable to pain of biopsy), injection site pain, dyspnea, skin pustules, rashes, cough, sputum, diarrhea, change in Eastern Cooperative Oncology Group (ECOG) performance status, hematology, or biochemistry that could not be attributed to another cause and was temporally related to the administration of VV-IL-2.

Evaluation of viral transmission

Tumor biopsies, urine, blood, mouth wash samples, sputum, and pleural fluid (where available) were obtained and sent for viral culture. Sera from patients and their close contacts were obtained and tested for the presence of anti-VV immunoglobulin G (IgG) by an indirect immunofluorescent Ab assay.

Viral culture was performed using Vero cell cultures in 48-well trays. Specimens (100 μ L) were inoculated, and plates were centrifuged at 2000 $\times$ g for 1 hour at 37°C to enhance infectivity. This method had been tested to show its ability to support the growth of VV and VV-IL-2.

Vaccinia Ab assay

RK-13 cell monolayers in 96-well flat-bottom plates were infected with a dose of VV that was sufficient to produce discrete foci of infection. Serial 2-fold dilutions of the patient's sera were added, and Ab binding was detected using anti-human IgG fluorescein isothiocyanate conjugate (Silenus, Victoria, Australia) and ultraviolet microscopy.

Gene expression

Reverse transcriptase (RT) VV-IL-2 PCR was performed using VV-IL-2 sequence primers on tumor biopsies. Tumor biopsies were homogenized in the presence of RNAzol B (Tel-Test, Friendswood, Tex) using a polytron blender. Total RNA was extracted according to the RNAzol B protocol. Up to 5 μ g of total RNA was reverse transcribed in the presence of ribonuclease inhibitor (40 U) using oligo(dT)₁₅₋₁₈ (500 ng)

and 12.5 U of avian Moloney virus RT (Promega, Madison, Wis) at 42°C for 1 hour. PCR was performed on a thermocycler (Perkin-Elmer, Norwalk, Conn) using oligonucleotide primers specific for human IL-2 (5'-ATG TAC AGG ATG CAA CTC CTG TCT T-3') and 3' of the *Eco*RI site in the VV-TK gene (5'-GTC CCA TCG AGT GCG GC-3'). The reaction mixture contained 10 pmol of each primer, 1.6 mM deoxynucleoside triphosphates, 2.0 mM Mg²⁺, and 0.2 U of *Taq* polymerase (Promega). The PCR consisted of 1 cycle of denaturation at 94°C (4 minutes), annealing at 55°C (1 minute), and extension at 72°C (2 minutes). This was followed by 49 cycles of denaturation at 94°C (30 seconds), annealing at 55°C (1 minute), and extension at 72°C (2 minutes). PCR products were visualized by ethidium bromide staining after electrophoresis through 1.2% (wt/vol) agarose. A glyceraldehyde-3-phosphate dehydrogenase internal control was amplified from all cDNA samples using specific primers (sense, 5'-GAA GGT CGG TGT GAA CGG ATT-3'; antisense, 5'-CGG AAG GGG CGG AGA TGA TGA-3'). The negative control RNA from activated human blood T lymphocytes was tested using these primers before testing patient tumor biopsy samples and during the study; this RNA remained negative.

Tumor biopsy immunohistochemical staining

Frozen sections of tumor biopsies were obtained, fixed in 1% paraformaldehyde, and washed; endogenous peroxidase was blocked by 1% hydrogen peroxide. Primary Abs CD3, CD4, and CD8 (Dako, Carpinteria, Calif) were then applied, followed by the secondary Ab, biotinylated sheep anti-mouse Ig (Jackson Laboratories, West Grove, Penn), and the addition of streptavidin-horseradish peroxidase (Dako). Staining was visualized by precipitating 3,3 diaminobenzidine (Sigma, St. Louis, Mo) and counterstaining with hematoxylin.

RESULTS

In vitro production of IL-2

The two human MM cell lines (JU77 and NO36) infected with VV-IL-2 produced significant amounts of IL-2 at an MOI of 0.5, ranging from 1192 to 3767 ng/mL at 24 hours (Fig 1). These data are consistent with the known kinetics of VV-IL-2 infection of cells, with maximal production of IL-2 usually seen at 24 hours postinfection.⁸

Patient characteristics

Six patients were administered VV-IL-2 i.t., and their characteristics are summarized in Table 1. In brief, patients were between 55 and 71 years of age; five were male and one was female; all had an ECOG performance status of 1 and a mean of 16 months from MM diagnosis (range 8–27 months). Patients had between 2 and 12 treatments (mean 5 treatments).

Toxicity

No toxicity was observed. There was no change in hematological or biochemical parameters in any patient during the course of treatment.

Figure 1. *In vitro* production of IL-2 from two human mesothelioma cell lines (JU77 and NO36) after infection with VV-IL-2 at an MOI of 0.5 at 24 and 72 hours. After 72 hours, all cells were infected; a later timepoint would not have provided accurate data at this MOI.

Transmission

VV-IL-2 excretion. VV-IL-2 was not cultured from sputum, urine, mouth wash samples, pleural fluid (obtained only for patient 6), or blood in any patient. The tumor biopsies from patient 4 were positive on culture for VV on all three samples taken during the second cycle of treatment. All other patients' tumor biopsies were negative for VV on culture.

Clinical features and Ab titers of contacts. A total of 31 contacts of the 6 patients were evaluated. None of these contacts developed any signs or symptoms of VV infection at any stage after exposure to a patient. None of the unvaccinated contacts showed any increase in serological titer to VV after exposure to a patient receiving i.t. VV-IL-2.

Immune response to VV in patients

VV IgG titers increased progressively with treatment in all patients, from a minimum titer of 1/10 to a maximum titer of 1/1280, indicating viral protein production *in vivo* (Fig 2). The exception was patient 1, who had high titers before treatment due to revaccination with VV.

Table 1. Patient Characteristics

Patient number	Age (years)	Sex	Performance status (ECOG)	Months since diagnosis	Number of treatments administered
1	57	Male	1	8	4
2	66	Male	1	17	4
3	55	Female	1	27	3
4	65	Male	1	16	2
5	61	Male	1	19	7
6	71	Male	1	9	12

Viral gene expression

VV-IL-2 mRNA was detected by RT-PCR (50 cycles) in tumor biopsies for up to 3 weeks after VV-IL-2 injection, as shown in Figure 3. This shows that in patients 1 through 4, the percentage of total positive VV-IL-2 mRNA tumor biopsies was greatest on days 1–3 (67% positive) after VV-IL-2 injection; this percentage subsequently decreased by days 5–8 (54% positive) and day 21 just before the next injection (40% positive). Contingency table analysis indicated no statistically significant difference between the three timepoints ($P = .46$). Similarly in patients 5 and 6, 63% of tumor biopsies were positive for VV-IL-2 mRNA on day 1 after VV-IL-2 injection. Figure 4 illustrates that VV-IL-2 mRNA was detectable in tumor biopsies regardless of anti-VV IgG titer. Contingency table analysis indicated that there was no statistically significant difference between the two groups ($P = .41$). This suggests that the presence of high levels of anti-VV Abs does not influence the pattern of expression of VV-IL-2 mRNA in tumor tissue.

T lymphocytes within tumor biopsies

A total of 36 tumor biopsies were available for immunohistochemistry staining at different timepoints postinjection. Of these, 18 showed staining with CD3, a nonspecific T-cell marker, when compared with pretreatment controls. Satisfactory staining with CD4 and CD8 could not be obtained due to limited section material.

Clinical responses

No significant tumor regression was observed either radiologically or clinically.

Figure 2. Anti-VV IgG titers in six patients receiving VV-IL-2 therapy. In all patients, IgG titers were measured at baseline (day 0) and just before each VV-IL-2 injection. In addition, IgG titers were measured on days 3 and 8 of each treatment cycle in patients 1 and 2, on days 1 and 7 of each treatment cycle in patients 3 and 4, and on day 1 of each treatment cycle in patients 5 and 6.

DISCUSSION

This is the first study using vaccinia-cytokine constructs in patients with solid malignancy; it has resulted in the surprising finding that the use of VV as a vector is associated with relatively prolonged viral gene expression within tumors after repeated injections despite the presence of systemic humoral immunity.

MM is an untreatable tumor that is considered to be an ideal target disease for gene therapy approaches, particularly when seen in the context of a progressive

increase in the incidence of this tumor worldwide.^{18,19} Evidence that MM may be susceptible to immunotherapeutic approaches has come from a number of laboratories worldwide, and we have good evidence that the production of cytokines within MM deposits induces strong anti-MM immunity. We selected the IL-2 gene for our initial experiments because it had powerful effects in our animal model¹⁹ and its effects in human cancer clinical trials have been well-described.^{2,20-24}

Our initial task was to evaluate the toxicity of this VV-cytokine construct. Although unmodified VV has

Figure 3. Percentage of tumor biopsies expressing VV-IL-2 in patients 1 through 4. Measurements were performed on days 1-3, days 5-8, and day 21 of each cycle after VV-IL-2 injection, using PCR primers specific for VV-derived IL-2.

Figure 4. Percentage of tumor biopsies expressing VV-IL-2 (measured as described in the legend to Fig 3) and anti-VV immune status in all six patients. Low Ab titer was defined as an anti-VV Ab titer of $\leq 1/80$; high Ab titer was defined as an anti-VV Ab titer of $>1/80$.

been injected intralesionally into patients with cancer in the past, most of those patients had cutaneous malignancies.²⁵⁻²⁸ We were not certain that our construct would not be widely disseminated and cause systemic toxicities, either due to the VV itself or to the IL-2 transgene. No systemic toxicities were noted. The first patient, who underwent boosting vaccination, exhibited transient fevers after VV-IL-2 injections at the highest doses, but these occurred within the first 12 hours and were possibly due to vaccinia Ag-Ab formation rather than due to either IL-2 or any antitumoral immune response. No evidence of organ dysfunction was noted clinically or on laboratory analysis, even in patients with no immunity or low-level anti-VV Ab titers at the time of their first injection. Thus, it appears that with the dose of VV-IL-2 used (10^7 PFU), this approach is free of side effects. Substantial attention was paid to the VV-IL-2 injection site because of the anticipation that a skin lesion would form at the injection site, providing a potential source of transmission to contacts. The fact that no patient developed a s.c. lesion presumably partly reflected the injection technique (injection deep into the tumor tissue) and partly the disabled nature of the virus, which limited its capacity to replicate in normal tissues such as the epidermis (see below). Importantly, virus was not detected in bodily secretions, such as urine and sputum, or detected in blood samples. These observations, taken together with the lack of change in anti-VV Ab titers in contacts, confirm that there is little risk of transmission of this agent to contacts of patients undergoing this type of therapy. This is an important observation, as it enables patients to undergo outpatient therapy rather than inpatient care, which is expensive and restrictive for the patient.

We chose a direct intralesional approach for the following reasons. First, distal approaches largely require the use of autologous cell lines; however these can only be generated in ~25-30% of patients with solid malignancy, so the use of direct intralesional approaches bypasses that requirement. Second, direct injection over-

comes the problems of targeting the cytokine to the tumor tissue. Third, we reasoned that the local generation of a specific antitumoral response could potentially induce some level of specific reactivity, breaking tolerance to tumor Ags and inducing reactivity at other sites that are distal to the site of injection. Overall, this approach avoids systemic and intracavity routes, which have potential side effects, and maximizes the chances of generating a high level of gene expression within the tumor itself.

Of critical importance in this study was the determination of the duration and level of gene expression after each VV-IL-2 injection. MM is an ideal tumor to study in this regard, as it is very accessible. Indeed, we limited our pilot study to patients whose tumor had extended between the ribs to form a s.c. deposit, that is, patients in whom we could inject the vector accurately, monitor tumor size directly, and biopsy the injection site with reasonable accuracy. This also minimized the requirement for serial CT scans. PCR techniques provided us with an opportunity to measure the IL-2 mRNA produced by the VV-IL-2 as distinct from endogenous IL-2. Unfortunately, we cannot be certain that in all cases the tumor biopsies were from the exact site of VV-IL-2 injection, and we cannot estimate the exact distribution or spread of infection around the injection site. It is possible that more cells were actually transduced but were not satisfactorily sampled. However, our decision to focus on s.c. tumor nodules for injection and sampling no doubt markedly increased the likelihood that our sampling procedures were reasonably accurate (in contrast to approaches requiring CT scan guidance for access to deeper tissues). One of the potential advantages of this vector is that the vector may be rendered replication-competent using the TK region of host tumor cells for replication and may actually spread beyond the original site of injection.

As expected, in patients with very low levels of anti-VV immunity, VV-IL-2 mRNA expression was maximal on the first day after injection and then reduced to

background over ~1–3 weeks. To our surprise however, a similar pattern was noted in patients whose anti-VV immunity had been boosted. In contrast, when VV-Ag recombinants were used in vaccination studies, systemic immunity limited viral persistence in skin tissues.²⁸ This suggests that in our study, the disabling of the VV by the insertion of the IL-2 gene into the TK region altered the growth pattern sufficiently to prevent lysis of the tumor cell, thereby maintaining the VV-IL-2 in intracellular locations where it was not accessible to circulating Abs. Presumably, any anti-VV cytotoxic T lymphocytes were not able to rapidly lyse VV-infected cells within the tumor milieu. These unanticipated observations are encouraging and suggest that VV-IL-2, modified in this way, may represent a good vector for gene transfer. In fact, by disabling the VV by inserting the IL-2 gene into the TK region, the VV will be capable of limited replication in tumor cells, relying on the TK produced endogenously by the tumor cells, whereas it is unlikely to replicate at all in normal cells in which endogenous TK activity is low, adding support to the notion that it may be a useful cancer gene therapy vector.

Although VV-IL-2 mRNA gene expression persisted over relatively prolonged periods, the overall level of expression was modest. Nevertheless, some biological changes were induced within the tumor, manifest as variable increases in T-cell infiltrates at the site of injection. Of course there is no accurate means of determining whether or not this T-cell infiltrate is related to a specific host antitumoral response, due to nonspecific effects of IL-2, due to the presence of the VV alone, or due to some combination of the above. Although the ideal way to follow the immunobiological effects in this system would be to study specific antitumoral reactivities, there is no accurate way to do this in most solid human tumor systems when there are no clearly defined tumor Ags. Cutaneous delayed-type hypersensitivity tests, cytotoxic T-lymphocyte generation, and Western blotting have all been used with variable levels of reproducibility. Using Western blotting and cDNA library screening techniques, we have recently identified some putative MM Ags; in future gene therapy studies in MM, we will attempt to follow host anti-MM reactivity using these techniques.²⁹

In this limited study, no tumor regression was noted. Although evaluation of antitumoral effects was not the main aim of this study, the lack of responses in this cohort of patients plus the modest increase in lymphocyte infiltrates in the treated tumors suggests that we have yet to achieve the optimum therapeutic regime using this approach to gene therapy. In subsequent studies, we plan to continue to increase the dose, incorporating multiple genes including IL-2, IL-12, and/or granulocyte-macrophage colony-stimulating factor, cytokines which have proven to be the most powerful inducers of anti-MM responses in our murine model and have the potential to act synergistically.

In conclusion, this VV-cytokine construct, when injected directly into a solid tumor, is associated with prolonged levels of viral-derived cytokine mRNA re-

gardless of the presence of a systemic antiviral humoral immune response, supporting the notion that the use of VV as a cytokine vector has promise as an immunological gene therapy approach in patients with solid malignancy.

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