Treatment of Experimental Subcutaneous Human Melanoma with a Replication-Restricted Herpes Simplex Virus Mutant

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Modified, non-neurovirulent herpes simplex viruses (HSV) have shown promise for the treatment of brain tumors, including intracranial melanoma. In this report, we show that HSV-1716, an HSV-1 mutant lacking both copies of the gene coding-infected cell protein 34.5 (ICP 34.5), can effectively treat experimental subcutaneous human melanoma in mice. *In vitro*, HSV-1716 replicated in all 26 human melanoma cell lines tested, efficiently lysing the cells. Therapeutic infection of subcutaneous human melanoma nodules with HSV-1716 led to viral replication that was restricted to tumor cells by immunohistochemistry. Moreover, HSV-1716 treatment significantly inhibited progression of preformed subcutaneous human melanoma nodules in SCID mice and caused complete regression of some tumors. This work expands the potential scope of HSV-1–based cancer therapy.

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The incidence of melanoma has been increasing worldwide for the last 20 y (Balch *et al*, 1983). The Centers for Disease Control recently reported that the incidence of melanoma in the United States increased about 4% per year between 1973 and 1991 with a concomitant 34% rise in death rate (Anonymous, 1995). At present, advanced (stage 4) melanoma is refractory to all standard cancer therapies (Johnson *et al*, 1995).

Viral-based treatment of tumors has been reported in experimental systems with parvovirus H-1, Newcastle disease virus, retroviral vectors containing drug susceptibility genes, and herpes simplex type I virus (HSV-1) (Dupressoir *et al*, 1989; Martuza *et al*, 1991; Markert *et al*, 1992; Reichard *et al*, 1992; Ram *et al*, 1993; Takamiya *et al*, 1993). The mechanisms by which viruses improve the outcome in experimental tumor systems are complex and incompletely understood. As is the case for host cells during a natural infection, tumor cells infected during a therapeutic viral infection can be damaged or killed directly by the virus or by immune responses to the virus.

Published uses of modified, replicating HSV to treat cancer have centered around tumors of central nervous system origin (Martuza *et al*, 1991; Markert *et al*, 1993; Boviatsis *et al*, 1994; Jia *et al*, 1994; Mineta *et al*, 1994; Randazzo *et al*, 1995). HSV-1716 is a mutant that has a 759-bp deletion in the genes coding for infected cell protein 34.5 (ICP 34.5) (MacLean *et al*, 1991). This mutation, through mechanisms that are unclear, severely attenuates the ability of HSV-1716 to replicate in normal tissues (MacLean *et al*, 1991; Robertson *et al*, 1992; Spivack *et al*, 1995) but does not appear to affect the virus' ability to replicate in malignant cells (Randazzo *et al*, 1995; Mckie *et al*, 1996; Kucharczuk *et al*, 1997). The virulence of HSV-1716 is extremely attenuated in animal models of HSV pathogenesis (Valyi-Nagy *et al*, 1994; Spivack *et al*, 1995). Moreover, we have shown that HSV-1716 replicates poorly in normal human skin grafted onto severe combined immunodeficient (SCID) mice and have used this virus to successfully treat intracranial tumors, including melanoma, and intraperitoneal human mesothelioma, in normal and immunodeficient mouse models (Kesari *et al*, 1995; Randazzo *et al*, 1995, 1996; Kucharczuk *et al*, 1997).

Because of (i) the lack of effective therapy for advanced melanoma, (ii) our success treating melanoma brain tumors, and (iii) the attenuated replication of HSV-1716 seen in normal tissues, we have initiated experiments to study HSV-1716-based therapy of subcutaneous human melanoma.

MATERIALS AND METHODS

Tumor Cells Human melanoma cell lines (a generous gift from Meenhard Herlyn, Wistar Institute, Philadelphia, PA) were isolated as previously reported (Juhasz *et al*, 1993). Lines 1205 and WM-451-Lu were derived from metastatic tumor nodules. Cells were grown in plastic flasks in AUTO-POW media containing penicillin, streptomycin, and 5% calf serum at 37°C in a humidified environment with 5% CO_2.

Virus Production To produce virus stocks, subconfluent monolayers of baby hamster kidney 21 clone 13 (BHK) cells were infected with HSV strains 1716 or wild type 17^+. Virus was concentrated from the culture and titrated by plaque assay (Spivack and Fraser, 1987). All viral stocks are stored frozen at −70°C and thawed rapidly just prior to use.

Production of UV-Inactivated Virus Ultraviolet inactivation of HSV was performed using the method of Notarianni and Preston (1982). After inactivation, the viral suspension was titered on BHK cells to confirm that

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Abbreviations: HSV, herpes simplex virus; MOI, multiplicity of infection; PFU, plaque-forming unit(s); ICP, infected cell protein; SCID, severe combined immunodeficient.

it could no longer establish a lytic infection, stored frozen at −70°C, and thawed rapidly just prior to use.

Viral Growth Kinetics Assay Subconfluent monolayers (5×10^5 cells in six-well plastic tissue culture plates) were infected with 5×10^3 plaque-forming units (PFU) of HSV in 1 ml of AUTO-POW media containing penicillin, and streptomycin. For the time 0 measurement, the cell monolayer was scraped off immediately into the viral inoculum suspension and frozen at −70°C. For the remaining time points, the viral inoculum was incubated on the cell monolayer at 37°C for 1 h with gentle rocking and then aspirated off. The infected monolayers were washed twice with media and resuspended in 1 ml of AUTO-POW media containing penicillin, streptomycin, and 5% calf serum. At the appropriate times post infection, the monolayers were harvested with a cell scraper, and the suspension was frozen at −70°C. Following two cycles of freezing and thawing, each sample was titrated by plaque assay on BHK cells (Spivack and Fraser, 1987).

Cell Viability Assay Cells were plated in 96 well plates at a density of 5×10^3 cells per well. Twenty four hours later the cells were infected with HSV-1716 at a multiplicity of infection (MOI; the ratio of plaque forming units of virus to cells) of 0, 0.01, 0.1, or 1.0, or HSV-17$^+$ at an MOI of 0.1. Viability was assessed at 24, 48, 72, and 96 h post infection (CellTiter 96 Aqueous Nonradioactive MTT Cell Proliferation Assay; Promega Corporation, Madison WI). The percent viability is defined as the ratio of the mean absorbance of six treated wells at 490 nm to the mean absorbance of six uninfected controls at each time point. Background absorbance, obtained by lysing uninfected cells with Triton X100 at a final concentration of 10% was subtracted from all experimental values prior to calculations.

Subcutaneous Tumor Production and Assays Mice were anesthetized with intramuscular ketamine/xylazine (87 mg ketamine/kg; 13 mg xylazine/kg). A patch of hair was removed from one flank using a chemical depilatory agent (Magic Shaving Powder, Carson Products Co., Savannah GA.) Subcutaneous injection of 1×10^5 of either 1205 or WM-451-Lu melanoma cells in a total volume of 50 μl was performed using a Hamilton syringe and a disposable 28 g needle. Tumor volumes assume a spherical tumor shape, and were calculated based on a radius obtained from orthogonal measurements of tumor diameters using a micrometer-caliper. For the experiments with 1205 melanoma, mice were randomly divided into three groups on day 14 post tumor cell injection. One group was treated with a 25 μl intratumoral injection of 2.5×10^6 PFU of HSV-1716 using a Hamilton syringe and a disposable 30 g needle. The second and third groups were injected, respectively, with an equal volume of UV-inactivated HSV-1716 or viral culture medium alone. For the experiments with WM-451-Lu melanoma, mice were randomly divided into two groups on day 28 post tumor cell injection. One group was treated with a 25 μl intratumoral injection of 2.5×10^6 PFU of HSV-1716, and the other group was injected with an equal volume of UV-inactivated HSV-1716.

Immunohistochemistry Viral antigen-expressing cells were detected by the indirect avidin-biotin immunoperoxidase method (Vector Labs, Burlingam, CA) as specified by the manufacturer with slight modifications developed in our laboratory (Gesser *et al*, 1994). Rabbit antiserum to HSV-1 (Dako Corp., Carpinteria, CA) was used at a dilution of 1:1000.

Statistical Analysis Data analysis, including calculations of means, standard deviations, and repeated measures analysis of variance (ANOVA), was performed using StatView statistical software (Abacus Concepts, Berkeley CA) on an Apple Macintosh computer (Cupertino, CA).

RESULTS

As an initial step in assessing the viability of HSV-based therapy of human melanoma, the efficiency of HSV-1716 replication was determined for a panel of human melanoma cell lines and compared to the replication of HSV-17$^+$, the parental strain from which HSV-1716 was derived. A representative experiment utilizing the 1205 melanoma cell line is shown in **Fig 1**. The kinetics of virus production were nearly identical for HSV-1716 and HSV-17$^+$. The replication cycle of HSV-1716 in these cells is approximately 24 h, and each replication cycle yields an approximately 4 log fold increase in lytically active virus.

To demonstrate the ability of HSV-1716 to directly kill human melanoma cells, we performed *in vitro* cell viability assays at various times following viral infection. A representative experiment utilizing the 1205 melanoma cell line is shown in **Fig 2**. At MOIs ranging from 0.01–1.0, HSV-1716 efficiently killed the tumor cells in a time- and dose-responsive fashion.

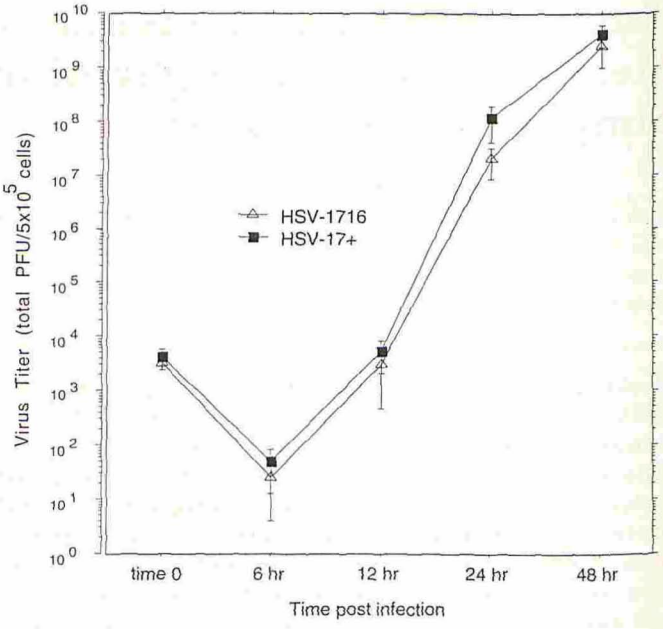

Figure 1. HSV-1716 and HSV-17$^+$ replicate efficiently in human melanoma cells *in vitro*. Subconfluent monolayers of 1205 cells were infected with 5×10^3 PFU of HSV-1716 or HSV 17$^+$. For the time 0 measurement, the cell monolayer was scraped off into the viral inoculum suspension immediately and frozen. For the remaining time points, the viral inoculum was incubated on the cell monolayer at 37°C for 1 h with gentle rocking and then aspirated off. The infected monolayers were washed twice with media and resuspended in 1 ml of AUTO-POW media containing penicillin, streptomycin, and 5% calf serum. At the appropriate times post infection, the monolayers were harvested with a cell scraper, and the suspension was frozen at −70°C. Following two cycles of freezing and thawing, each sample was titrated by plaque assay on BHK cells. The data shown represent the mean ± SD of triplicate determinations at each time point.

A total of 26 human melanoma cell lines were tested similarly for lytic replication of HSV, and all of these replicated HSV-1716 and HSV-17$^+$ efficiently *in vitro* (data not shown).

We next determined whether replication of HSV-1716 could be demonstrated *in vivo* in pre-formed subcutaneous human melanoma nodules growing in SCID mouse skin and, more importantly, evaluated whether replication of the virus was detectable in surrounding normal tissues. This was accomplished by immunohistochemical evaluation of treated tumors and surrounding tissues. As shown in representative sections in **Fig 3**, immunohistochemical staining of subcutaneous 1205 melanoma nodules with antibody to HSV at 5 d **(Fig 3*B,C*)** or 10 d **(Fig 3*D,E*)** after intratumoral injection of HSV-1716 demonstrates positive staining that is dispersed throughout a large area of the tumor. At these times a significant portion of the HSV-1716–treated tumor nodules are necrotic. Moreover, no staining is observed in normal murine tissues surrounding the tumor nodules in these and all other sections examined. No staining, and no significant necrosis occurred in the representative control tumor treated with UV-inactivated HSV-1716 and subjected to the full immunohistochemical protocol **(Fig 3*A*)**.

Finally, to definitively test the *in vivo* efficacy of this approach, we attempted therapy of pre-formed subcutaneous tumors. As shown in **Fig 4*A***, HSV-1716 significantly inhibits progression of preformed subcutaneous 1205 melanoma tumor nodules relative to control tumors treated with UV-inactivated virus ($p < 0.0001$; repeated measures ANOVA) and also relative to control tumors treated with culture medium alone ($p < 0.0001$; repeated measures ANOVA). In addition, it was found that UV-inactivated HSV-1716 had no effect on tumor progression relative to tumor nodules

injected with viral culture medium alone ($p = 0.8238$; repeated measures ANOVA). Of the ten mice bearing 1205 melanoma tumors treated with HSV-1716 in this representative experiment, three had complete involution of melanoma nodules such that no palpable tumors were present from day 21 on. As shown in **Fig 4*B***, HSV-1716 significantly inhibits progression of pre-formed subcutaneous WM-451-Lu melanoma tumor nodules relative to control tumors treated with UV-inactivated virus ($p = 0.0012$; repeated measures ANOVA), For HSV-1716–treated mice bearing WM-451-Lu melanoma, four of ten had complete regression of subcutaneous nodules by day 42. In these mice, regression was subsequently confirmed histologically (data not shown). In none of the HSV-1716–treated animals was there any mortality or morbidity noted.

DISCUSSION

In this report we demonstrate that the "replication- restricted" herpes simplex mutant HSV-1716 is a safe and effective therapy for pre-formed subcutaneous human melanoma nodules in SCID mice.

The virus used for our studies, called HSV-1716, has a 759-bp deletion in the ICP34.5 gene (MacLean *et al*, 1991). *In vitro*, ICP34.5 null HSV-1 mutants grow as well as wild-type virus on cells of most established cell lines (Chou *et al*, 1990; MacLean *et al*, 1991; Bolovan *et al*, 1994). On primary cells, however, these viruses show impaired replication (Bolovan *et al*, 1994). *In vivo*, ICP34.5 null HSV-1 mutants are relatively avirulent. They replicate marginally if at all within the central nervous system of immunocompetent mice and do not cause encephalitis (Chou *et al*, 1990; Taha *et al*, 1990; MacLean *et al*, 1991), in sharp contrast to wild-type HSVs, which grow exponentially in brain and kill mice within days of inoculation (MacLean *et al*, 1991). These mutants also show impaired or no replication in non-neuronal tissues of experimental animals, such as corneal epithelium and vaginal mucosa (Whitley *et al*, 1993; Spivack *et al*, 1995). We have shown that relative to wild-type HSV-17$^+$ and the revertant virus HSV-1716R, the replication of HSV-1716 is severely restricted in normal human skin xenografts growing on SCID mice (Randazzo *et al*, 1996). Moreover, an extremely sensitive PCR based assay failed to detect dissemination of HSV-1716 into any normal tissues following intraperitoneal infection of human mesothelioma–bearing SCID mice (Kucharczuk *et al*, 1997). Thus, there is considerable data from rodents showing that ICP 34.5 null HSV poses little risk to the host.

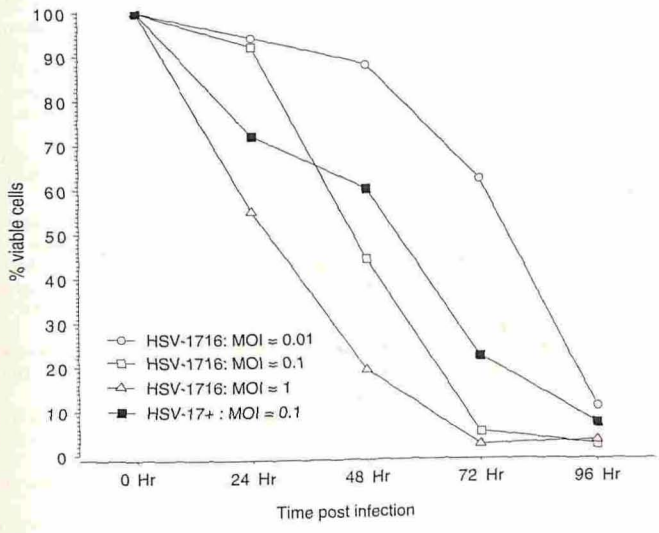

Figure 2. HSV-1716 efficiently kills human melanoma cells *in vitro*. Cells were plated in 96-well plates at a density of 5×10^3 cells per well. Twenty four hours later the cells were infected with HSV-1716 at an MOI of 0, 0.01, 0.1, or 1.0, or HSV-17$^+$ at an MOI of 0.1. Viability was assessed at 24, 48, 72, and 96 h post infection (CellTiter 96 Aqueous Nonradioactive MTT Cell Proliferation Assay; Promega Corporation, Madison WI). Each data point is derived from the mean of six identical wells. The percent viability is defined as: (absorbance-infected wells − background)/(absorbance uninfected wells − background) Background absorbance was obtained by lysing uninfected cells with Triton X100 at a final concentration of 10%.

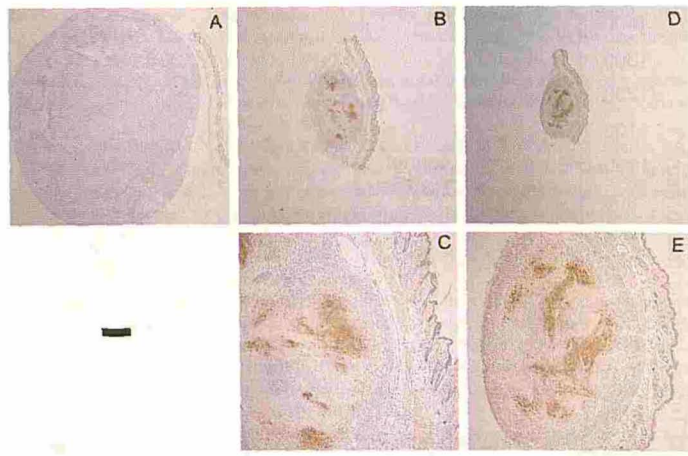

Figure 3. Replication of HSV-1716 is restricted to melanoma cells following injection into pre-formed subcutaneous tumor nodules. Subcutaneous tumors were produced by injection of 1×10^5 1205 melanoma cells into SCID mice. On day 14 post tumor cell injection, some mice were treated with an intratumoral injection of 2.5×10^6 PFU of HSV-1716, while control mice received an equivalent volume of UV-inactivated virus. **Figure 3*A*** shows a representative section from a control tumor treated with UV-inactivated 1716 and harvested 10 d later. **Figure 3*B,C*** shows representative low and higher power sections from a tumor treated with HSV-1716 and harvested 5 d later. **Figure 3*D,E*** shows representative low and higher power sections from a tumor treated with HSV-1716 and harvested 10 d later. The tumor and surrounding skin were excised, fixed, stained immunohistochemically for HSV-1, and counterstained with hematoxylin. All sections are oriented with the epidermis overlying the tumor nodule facing toward the *right* side of the panel. *Scale bar*, 1 mm *(panels A, B, D)*; 250 μm *(panels C, E)*.

The neurotropic nature of HSV suggests that neural crest-derived melanoma cells would be a good host cell for this virus. Our experience with murine melanoma, however, is that the majority of these cell lines are refractory to HSV infection (Randazzo *et al*, 1995; and B.P. Randazzo, unpublished observations). This is apparently not an issue with human melanoma because we now report that all 26 lines examined, derived from primary and metastatic patient material, supported brisk lytic replication of HSV.

Efficacious therapy of experimental melanoma has been demonstrated with suicide gene transfer using *in vitro* transfection and *in vivo* adenoviral mediated infection (Golumbek *et al*, 1992; Vile and Hart, 1993; Bonnekoh *et al*, 1995). These approaches have utilized the HSV thymidine kinase (HSV TK) gene to transfer susceptibility to ganciclovir to tumor cells. Although adenoviral based gene transfer typically results in transduction of only 1–2% of the tumor cells within a nodule, a 40–50% reduction in tumor growth rate was effected (Bonnekoh *et al*, 1995). Such reductions in tumor growth rate in the face of relatively inefficient gene transfer is ascribed to a poorly understood "bystander effect." HSV-1716 contains the very HSV TK gene utilized in the gene transfer experiments noted above. As shown in **Fig 3**, the efficiency of HSV-1716 spread through the experimental melanomas is very good and certainly far exceeds the 1–2% tumor cell transduction reported for adenovirus-based transduction following direct intratumoral injection. One could envision a therapeutic approach in which efficient infection of a tumor would be accomplished with a replication-restricted mutant such as HSV-1716 and followed up with systemic ganciclovir. Thus, direct lytic infection and killing of

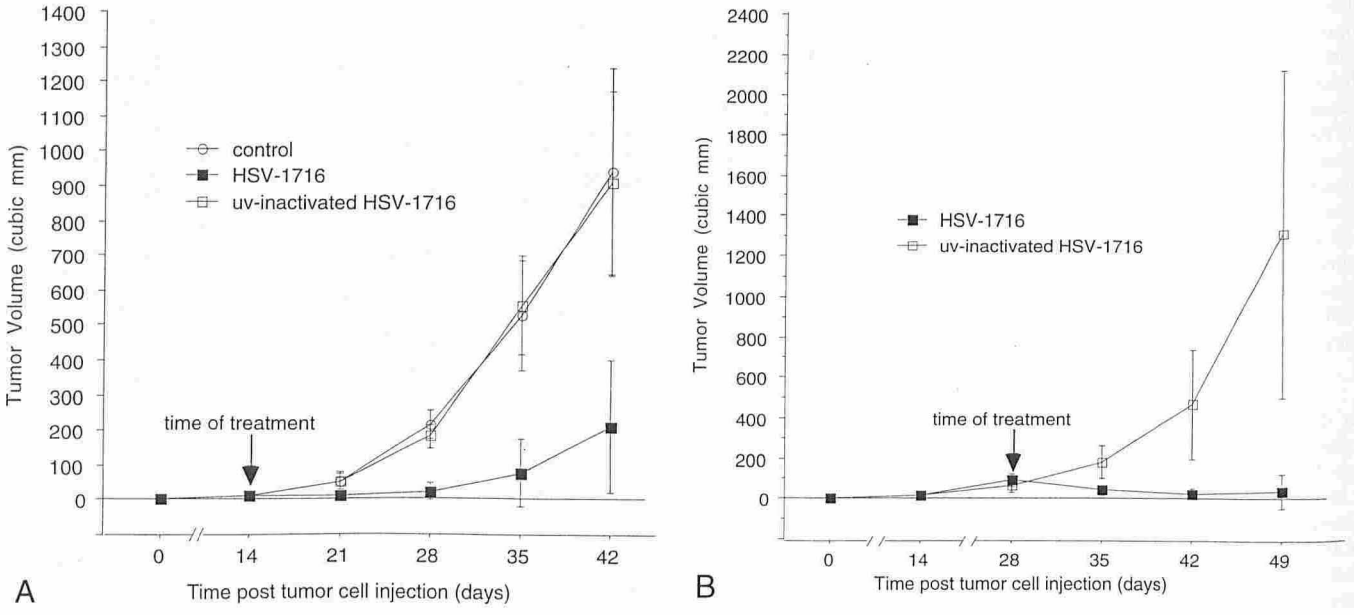

Figure 4. Intratumoral treatment of pre-formed subcutaneous melanoma with HSV-1716 causes a significant inhibition of tumor progression. For the experiment shown in *(A)*, anesthetized mice were injected subcutaneously with 1×10^5 1205 melanoma cells. On day 14 post tumor cell injection, mice were randomly divided into three groups. One group was treated with a 25 μl intratumoral injection of 2×10^6 PFU of HSV-1716, and animals in the two control groups were injected with either an equal volume of UV-inactivated 1716 or viral culture medium. The experiment presented is one of two performed, and both yielded essentially identical results. For the experiment shown in *(B)*, anesthetized mice were injected subcutaneously with 1×10^5 WM-451-Lu melanoma cells. On day 28 post tumor cell injection, mice were randomly divided into two groups. One group was treated with a 25 μl intratumoral injection of 2×10^6 PFU of HSV-1716, and animals in the control group were injected with an equal volume of UV-inactivated 1716. The data shown represent the mean $\pm$ SD of 10 mice at each point.

tumor cells would be augmented by a bystander effect to improve efficacy.

A requirement for injection of virus directly into tumor nodules is a potential limitation for treatment of widespread metastatic or occult disease, which is presently the most refractory form of melanoma. It is possible, however, that viral based tumor therapy could invoke a therapeutically beneficial vaccine effect that could augment the direct lytic effects seen *in vitro* and in a SCID/xenograft model system. It has been shown that infection of nonimmunogenic tumor cells with influenza virus, ectotropic retrovirus, or hybridization with allogeneic partner cells can induce immunologic rejection responses against the original unmodified tumor cells (Klein and Boon, 1993). Although the exact mechanisms of this effect are unclear, it is likely that it is a result of improved antigen presentation and or helper effects. We are presently investigating whether HSV therapy can induce a specific immune response directed against noninfected tumor cells. It is our hope that this will allow for extension of efficacy to untreated and, possibly, uninfected tumor cells, broadening the applicability of this approach.

There is clearly significant work remaining before the ultimate utility of HSV-based melanoma therapy can be determined. We view this study as an important proof of principle, which suggests the exciting potential of this therapeutic approach.

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REFERENCES

Anonymous: Deaths from melanoma United States, 1973–1992. *MMWR* 44:343–347, 1995

Balch CM, Soong S-j, Murad T M, Smith JW, Maddox WA, Durant JR: A multifactorial analysis of melanoma. IV. Prognostic factors in 200 melanoma patients with distant metastases (stage III). *J Clin Oncol* 1:126–132, 1983

Bolovan CA, Sawtell NM, Thompson RL: ICP 34.5 mutants of herpes simplex virus type 1 strain 17syn+ are attenuated for neurovirulence in mice and for replication in confluent primary mouse embryo cell cultures. *J Virol* 68:48–55, 1994

Bonnekoh B, Greenhalgh DA, Bundman DS, Eckhardt JN, Longley MA, Chen SH, Woo SL, Roop DR: Inhibition of melanoma growth by adenoviral-mediated HSV thymidine kinase gene transfer *in vivo*. *J Invest Dermatol* 104:313–317, 1995

Boviatsis EJ, Chase M, Wei MX, Tamiya TRK, Hurford J, Kowall NW, Tepper R I, Breakfield XO, Chiocca EA: Gene transfer into experimental brain tumors mediated by adenovirus, herpes simplex virus (HSV), and retrovirus vectors. *Hum Gene Therapy* 5:183–191, 1994

Chou J, Kern ER, Whitley RJ, Roizman B.: Mapping of herpes simplex virus neurovirulence to gamma 1 34.5, a gene nonessential for growth in culture. *Science* 250:1262–1266, 1990

Dupressoir T, Vanacker J-M, Cornelis JJ, Duponchel N, Rommelacre J: Inhibition by parvovirus H-1 of the formation of tumors in nude mice and colonies *in vitro* by transformed human mammary epithelial cells. *Cancer Res* 49:3203–3208, 1989

Gesser RM, Valyi-Nagy T, Fraser NW: Restricted herpes simplex virus type 1 gene expression within sensory neurons in the absence of functional B and T lymphocytes. *Virology* 200:791–795, 1994

Golumbek PT, Hamzeh FM, Jaffe EM, Levitsky H, Lietman PS, Pardoll DM: Herpes simplex-1 virus thymidine kinase gene is unable to completely eliminate live, nonimmunogenic tumor cell vaccines. *J Immunother* 12:224–230, 1992

Jia WW-G, McDermott M, Goldie J, Cynader M, Tan J, Tufaro, F: Selective destruction of gliomas in immunocompetent rats by thymidine kinase-defective herpes simplex virus type 1. *J Natl Cancer Inst* 86:1209–1215, 1994

Johnson TM, Smith JW, Nelson BR, Chang A.: Current therapies for cutaneous melanoma. *J Am Acad Dermatol* 32:689–707, 1995

Juhasz I, Albelda S, Elder D, Murphy G, Adachi K, Herlyn D, Valyi-Nagy I, Herlyn M: Growth and invasion of human melanomas in human skin grafted to immunodeficient mice. *Am J Pathol* 143:528–537, 1993

Kesari S, Randazzo BP, Valyi-Nagy T, Huang QS, Brown SM, MacLean AR, Lee VM-Y, Trojanowski JQ, Fraser NW: Therapy of experimental human brain tumors using a neuroattenuated herpes simplex virus mutant. *Lab Invest* 73:636–648, 1995

Klein G, Boon T: Tumor immunology: present perspectives. *Curr Opin Immunol* 5:719–725, 1993

Kucharczuk JC, Randazzo BP, Chang MY, Amin KM, Elshami AA, Sterman DH, Rizk NP, Molnar-Kimber KL, Brown SM, MacLean AR, Litzky LA, Fraser NW, Albelda SM, Kaiser LR: Use of a "replication-restricted" herpes virus to treat experimental human malignant mesothelioma. *Cancer Res*, 57:466–471, 1997

MacLean AR, Ul-Fareed M, Robertson L, Harland J, Brown SM: Herpes simplex virus

type 1 deletion variants 1714 and 1716 pinpoint neurovirulence-related sequences in Glasgow strain 17+ between immediate early gene 1 and the 'a' sequence. *J Gen Virol* 72:631–639, 1991

Markert JM, Coen DM, Malick A, Mineta T, Martuza RL: Expanded spectrum of viral therapy in the treatment of nervous system tumors. *J Neurosurg* 77:590–594, 1992

Markert JM, Malick A, Coen DM, Martuza RL: Reduction and elimination of encephalitis in an experimental glioma therapy model with attenuated herpes simplex mutants that retain susceptibility to acyclovir. *Neurosurgery* 32:597–603, 1993

Martuza RL, Malick A, Markert JM, Ruffner KI, Coen DM: Experimental therapy of human glioma by means of a genetically engineered virus mutant. *Science* 252:854–856, 1991

Mckie EA, MacLean AR, Lewis AD, Cruickshank G, Rampling R, Barnett SC, Kennedy PGE, Brown SM: Selective *in vitro* replication of herpes simplex virus type 1 (HSV-1) ICP34.5 null mutants in primary human CNS tumors - evaluation of a potentially effective clinical therapy. *Br J Cancer* 74:745–752, 1996

Mineta T, Rabkin SD, Martuza RL: Treatment of malignant gliomas using ganciclovir-hypersensitive, ribonucleotide reductase-deficient herpes simplex viral mutant. *Cancer Res* 54:3963–3966, 1994

Notarianni EL, Preston CM: Activation of cellular stress protein genes by herpes simplex virus temperature-sensitive mutants which overproduce immediate early polypeptides. *Virology* 123:113–122, 1982

Ram Z, Culver KW, Wallbridge S, Blaese RM, Oldfield EH: *In situ* retroviral-mediated gene transfer for the treatment of brain tumors in rats. *Cancer Res* 53:83–88, 1993

Randazzo BP, Kesari S, Gesser RM, Alsop D, Ford JC, Brown SM, MacLean AR, Fraser NW: Treatment of experimental intracranial murine melanoma with a neuro-attenuated Herpes Simpex virus-1 mutant. *Virology* 211:94–101, 1995

Randazzo BP, Kucharczuk JC, Litzky L, Kaiser L, Brown S, Maclean A, Albelda S, Fraser N: Herpes simplex 1716 — an ICP 34.5 mutant — is severely replication restricted in human skin xenografts *in vivo*. *Virology* 223:392–396, 1996

Reichard KW, Lorence RM, Cascino CJ, Peeples ME, Walter RJ, Fernando MB, Reyes HM, Greager JA: Newcastle disease virus selectively kills human tumor cells. *J Surg Res* 52:448–453, 1992

Robertson LM, MacLean AR, Brown SM: Peripheral replication and latency reactivation kinetics of the non-neurovirulent herpes simplex virus type-1 variant 1716. *J Gen Virol* 73:967–970, 1992

Spivack JG, Fareed MU, Valyi-Nagy T, Nash TC, O'Keefe JS, Gesser RM, McKie EA, MacLean AR, Fraser NW, Brown SM: Replication, establishment of latent infection, expression of the latency-associated transcripts and explant reactivation of herpes simplex virus type 1 gamma 34.5 mutants in a mouse eye model. *J Gen Virol* 76:321–332, 1995

Spivack JG, Fraser NW: Detection of herpes simplex type 1 transcripts during latent infection in mice. *J Virol* 61:3841–3847, 1987

Taha MY, Brown SM, Clements GB, Graham DI: The JH2604 deletion variant of herpes simplex virus type 2 (HG52) fails to produce necrotizing encephalitis following intracranial inoculation of mice. *J Gen Virol* 71:1597–1601, 1990

Takamiya Y, Short MP, Moolten FL, Fleet C, Mineta R, Breakefield XO, Martuza RL: An experimental model of retrovirus gene therapy for malignant brain tumors. *J Neurosurg* 79:104–110, 1993

Valyi-Nagy T, Fareed MU, O'Keefe JS, Gesser RM, MacLean AR, Brown SM, Spivack JG, Fraser NW: The HSV-1 strain 17+ gamma 34.5 deletion mutant 1716 is avirulent in SCID mice. *J Gen Virol* 75:2059–2063, 1994

Vile RG, Hart IR: Use of tissue-specific expression of the herpes simplex virus thymidine kinase gene to inhibit growth of established murine melanomas following direct intratumoral injection of DNA. *Cancer Res* 53:3860–3864, 1993

Whitley RJ, Kern ER, Chatterjee S, Choi J, Roizman B: Replication, establishment of latency, and induced reactivation of herpes simplex virus gamma 1 34.5 deletion mutants in rodent models. *J Clin Invest* 91:2837–2843, 1993