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Oncolytic virus therapy using genetically engineered herpes simplex viruses

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1. ABSTRACT

Genetically engineered, conditionally replicating herpes simplex viruses type 1 (HSV-1) are promising therapeutic agents for cancer. They can replicate *in situ*, spread, and exhibit oncolytic activity via a direct cytocidal effect. In addition, oncolytic HSV-1 can transfer and express foreign genes in host cells. The phase I clinical study with G207, a double-mutated HSV-1, in recurrent malignant glioma patients has shown that oncolytic HSV-1 can be safely administered into human brains. The therapeutic benefits of oncolytic HSV-1 depend on the extent of both intratumoral viral replication and induction of host antitumor immune responses. We develop new-generation oncolytic HSV-1 by enhancing these properties while retaining the safety features. G47Δ was created from G207 by introducing another genetic mutation. Compared with G207, G47Δ showed 1) better stimulation of human antitumor immune cells, 2) better growth properties leading to higher virus yields and increased cytopathic effect *in vitro,* 3) better antitumor efficacy in both immuno-competent and -incompetent animals, and 4) preserved safety in the brain of HSV-1-sensitive mice. Preparation is under way for a clinical trial using G47Δ in progressive glioblastoma patients. G47Δ is also suited as a backbone vector for expressing foreign molecules. Using bacterial artificial chromosome and two DNA recombinases, we have created an "armed" oncolytic HSV-1 generation system that allows insertion of transgene(s) into the genome of G47Δ in a rapid and accurate manner. We found that expression of immunostimulatory molecules can significantly enhance the antitumor efficacy of G47Δ. Based on these advances, we anticipate that oncolytic virus therapy using oncolytic HSV-1 will soon be established as an important modality of cancer treatment.

2. INTRODUCTION

Oncolytic virus therapy is an attractive means of treating cancer (1). Viruses, especially herpes simplex virus type 1 (HSV-1) and adenoviruses, are genetically engineered to restrict virus replication to tumor cells and to widen the therapeutic window. Infected tumor cells are destroyed by a direct oncolytic activity of the viruses, and the recombinant viruses do not harm normal tissues. Oncolytic virus vectors can also be used for transgene delivery.

HSV-1, especially in comparison with adenovirus, has suitable features for cancer therapy: (i) HSV-1 infects most tumor cell types. (ii) A relatively low multiplicity of infection (MOI) is needed for total cell killing. (iii) Anti-viral drugs are available. (iv) A large genome (~152 kb) allows the insertion of large and/or multiple transgenes. (v) The host immune reactions enhance antitumor effects. (vi) Circulating anti-HSV-1 antibody does not affect cell-to-cell spread of the virus. (vii) There are HSV-1 sensitive mouse and nonhuman primate models for preclinical evaluation. (viii) Viral DNA is not integrated into the host genome.

3. G207 – A SECOND-GENERATION ONCOLYTIC HSV-1

For HSV-1, the principle of how to target viral replication to tumor cells is to inactivate or delete viral genes that are essential for viral replication in normal cells but dispensable in tumor cells, using features common for all types of cancer. Oncolytic HSV-1 therefore can be applied to a wide variety of cancer. The key to successful and practical development of oncolytic HSV-1 is the safety, *i.e.*, a wide therapeutic window, which can be achieved only via genetic engineering.

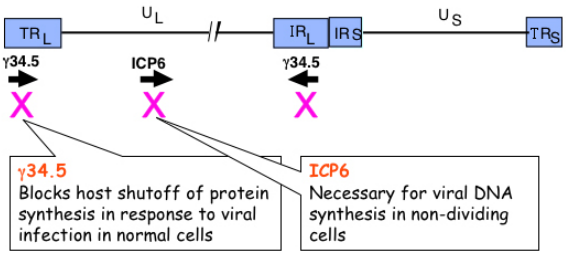

Figure 1. Structures of G207. The HSV-1 genome consists of long and short unique regions (U_L and U_S) each bounded by terminal (T) and internal (I) repeat regions (R_L and R_S). G207 was engineered from wild-type HSV-1 strain F by deleting 1 kb within both copies of the *γ34.5* gene, and inserting the E.coli *lacZ* gene into the ICP6 coding region.

G207 is the first oncolytic HSV-1 used in a clinical trial in the United States (2). This second-generation oncolytic HSV-1 has double mutations created in the HSV-1 genome (Figure 1). G207 has deletions in both copies of the *γ34.5* gene, the major determinant of HSV-1 neurovirulence (3). *γ34.5*-deficient HSV-1 vectors are considerably attenuated in normal cells, but retain their ability to replicate within neoplastic cells. In normal cells, HSV-1 infection induces activation of double-stranded RNA-dependent protein kinase (PKR), which in turn leads to phosphorylation of the alpha-subunit of eukaryotic initiation factor 2 (eIF-2α) and a subsequent shutdown of host and viral protein synthesis (4). The product of the *γ34.5* gene antagonizes this PKR activity. However, tumor cells have low PKR activities, thereby allowing *γ34.5*-deficient HSV-1 vectors to replicate (5, 6). G207 also has an insertion of the E. coli *lacZ* gene in the infected-cell protein 6 (*ICP6*) coding region (UL39), inactivating ribonucleotide reductase, a key enzyme for viral DNA synthesis in non-dividing cells but not in dividing cell (7).

G207 has been tested in more than 60 different cell lines and proved effective in all human tumor cell lines except for those derived from bone marrow (8). In human glioma and malignant meningioma cell lines, for example, G207 can achieve cell destruction of the entire cell population within 2 to 6 days at an MOI of 0.1 (2, 9). Whereas rodent cells are generally less susceptible to HSV-1 infection than human cells, G207 can destroy the entire cell population of N18 murine neuroblastoma cells within 3 days at an MOI of 1 (10). G207 manifests no effect on primary cultures of rat cortical astrocytes or cerebellar neurons even at 9 days post-infection, whereas, at the same MOI, the wild type HSV-1, the parental strain F, kills these normal cells (2). This difference in G207 cytopathic effect observed *in vitro* between tumor cells and normal cells is directly reflected in the results of *in vivo* studies.

In A/J mice harboring established syngeneic N18 tumors subcutaneously or in the brain, a single intraneoplastic inoculation with G207 caused a significant reduction in tumor growth or prolongation of survival (10). The most remarkable finding with G207 when tested in immuno-competent animals was that it induced systemic antitumor immunity (10, 11). In A/J mice bearing bilateral subcutaneous N18 tumors, intraneoplastic G207 inoculation into the left tumor alone caused growth reduction not only of the inoculated tumors but also of the non-inoculated contralateral tumors. The antitumor immunity was associated with an elevated cytotoxic T lymphocyte (CTL) activity specific to N18 tumor cells that persisted for at least 13 months.

An extensive *in vivo* safety evaluation was performed with G207 (12-15). The results are summarized as follows: 1) G207 does not cause disease in HSV-1-susceptible mice after intracerebral, intra-ventricular, intravenous, intraprostatic or intrahepatic injection at 10^7 plaque-forming units (pfu). 2) G207 does not cause disease in HSV-susceptible primates (Owl monkeys; *Aotus nancymae*) after intracerebral or intraprostatic injections of 1-3 x 10^7 or 10^9 pfu. 3) No elicitation of severe inflammatory response was observed with multiple inoculations or in animals with prior HSV-1 exposure. 4) No detectable reactivation of 'latent' HSV-1 in the brain was observed in Balb/c mice. 5) No detectable shedding of virus was observed after intracerebral inoculation in Owl monkeys. 6) Induction of anti-HSV antibodies was observed after intracerebral or intraprostatic injection in Owl monkeys. 7) G207 DNA was detectable in the brain up to 2 years after intracerebral injection and in the urethra, spleen and lymph nodes after intraprostatic injection in Owl monkeys.

4. G207 CLINICAL TRIAL

The G207 phase I clinical trial was performed between 1998 and 2000 at two institutions in the United States, Georgetown University Medical Center and University of Alabama at Birmingham (16). We treated 21 patients with recurrent malignant glioma: 16 patients with glioblastoma and 5 patients with anaplastic astrocytoma. G207 was administered directly into the tumor via stereotactic inoculation. This dose escalation study started from 10^6 pfu and increased to 3 x 10^9 pfu, with three patients at each dose. No acute, moderate to severe adverse events attributable to G207 were observed. Minor adverse events included seizure (2 cases) and brain edema (1 case). An improvement in Karnofsky score was observed in 6 of 21 patients (29%) at some time after G207 inoculation. Eight of 20 patients that had serial MRI evaluations had a decrease in tumor volume (enhancing area) between 4 days and one month post-inoculation. Patient #4 that received 10^7 pfu showed continuous decrease in tumor size after G207 inoculation, but died from irrelevant cerebral infarction 10 months after treatment. The trial proved the safety of G207 inoculated intratumorally in the brain up to 3 x 10^9 pfu.

5. G47Δ - A THIRD-GENERATION ONCOLYTIC HSV-1

We further developed a third-generation oncolytic HSV-1 termed G47Δ (Figure 2). G47Δ was newly created from G207 by introducing another genetic alterarion, *i.e.*,

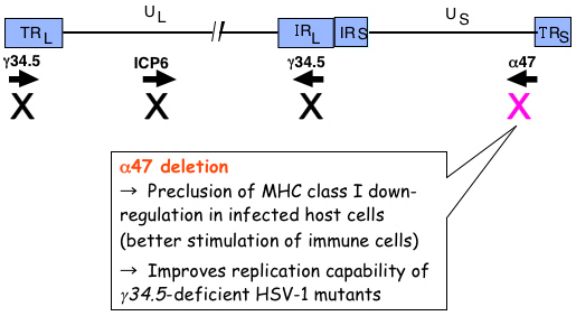

Figure 2. Structure of G47Δ. G47Δ was created from G207 by further deleting 312 bp within the *α47* gene. The deletion also places the *US11* gene under control of the *α47* immediate-early promoter.

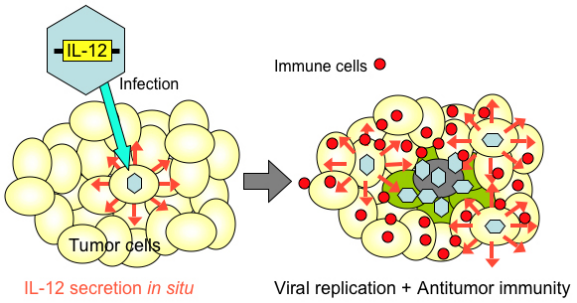

Figure 3. Concept of cancer therapy using oncolytic HSV-1 armed with an immunostimulatory gene. When oncolytic HSV-1 armed with the interleukin 12 (IL-12) gene infects tumor cells, IL-12 is secreted in the course of viral replication and stimulates the immune cells. In addition to direct tumor cell killing via viral replication and spread, tumor cells are destroyed by augmented antitumor immune responses, resulting in enhanced antitumor activities.

the deletion of the *α47* gene and the overlapping *US11* promoter region, in the G207 genome (17). Because the *α47* gene product (ICP47) inhibits transporter associated with antigen presentation (TAP), which translocates peptides across the endoplasmic reticulum, the down-regulation of MHC class I that normally occurs in human cells after infection with HSV-1 does not occur when the *α47* gene is deleted (18). G47Δ-infected human cells in fact presented higher levels of MHC class I expression than cells infected with other HSV-1 vectors (17). Further, human melanoma cells infected with G47Δ were better at stimulating their matched tumor-infiltrating lymphocytes *in vitro* than those infected with G207. The deletion also places the late *US11* gene under control of the immediate-early *α47* promoter, which results in suppression of the reduced growth phenotype of *γ34.5*-deficient HSV-1 mutants including G207 (19). In the majority of cell lines tested, G47Δ replicated better than G207, resulting in the generation of higher virus titers, and exhibiting greater cytopathic effect (17). In athymic mice bearing subcutaneous U87MG human glioma and A/J mice bearing subcutaneous Neuro2a neuroblastoma, G47Δ was significantly more efficacious than G207 at inhibiting the tumor growth when inoculated intraneoplastically (17). G47Δ was also more efficacious than G207 in athymic mice bearing intracerebral U87MG tumors (Ino Y, *et al.* unpublished data). Nevertheless, the safety of G47Δ remained unchanged from G207 following injection into the brain of HSV-1-sensitive A/J mice (17). Thus, with G47Δ, by creating the third engineered mutation within the G207 genome, we improved the efficacy of G207 without compromising its safety. G47Δ has been shown efficacious in animal tumor models of a variety of cancers including brain tumors, prostate cancer, breast cancer and schwannoma (17, 20-22). A phase I-II clinical trial using G47Δ in patients with progressive glioblastoma is underway at the University of Tokyo.

6. "ARMED" ONCOLYTIC HSV-1

One of the advantages of HSV-1 is the capacity to incorporate large and/or multiple transgenes within the viral genome. Certain antitumor functions may be added to oncolytic activities of recombinant HSV-1. Conventional homologous recombination techniques had required time-consuming processes to create "armed" oncolytic HSV-1. We recently established an innovative "armed" oncolytic HSV-1 construction system utilizing bacterial artificial chromosome (BAC) and two DNA recombinase systems (Cre/loxP and FLP/FRT) (23). Using G47Δ as the backbone, this system allows a rapid generation of multiple vectors with desired transgenes inserted in the deleted *ICP6* locus.

Aside from the extent of replication capability within the tumor, the efficacy of an oncolytic HSV-1 depends on the extent of antitumor immunity induction (10, 11). Therefore, while any transgene that does not interfere with HSV-1 replication may be used (24), the genes of immunomodulatory molecules would be reasonable candidates for "arming" oncolytic HSV-1. Using this construction system, we first created oncolytic HSV-1 armed with immunostimulatory genes such as interleukin 12 (IL-12), IL-18, or soluble B7-1. Immunostimulatory functions should augment the antitumor immunity induction that adds to direct oncolytic activity of the virus, resulting in enhanced antitumor activities (Figure 3). Using A/J mice bearing bilateral subcutaneous Neuro2a tumors, known to be poorly immunogenic, we tested the efficacy of a third-generation oncolytic HSV-1 armed with mouse fusion-type IL-12, termed T-mfIL12. When the viruses were inoculated into the left tumor only, T-mfIL12 showed a significantly better antitumor activity than the unarmed control virus, T-01, not only in the inoculated left tumors but also in the non-inoculated remote tumors (Miyamoto S, *et al.* unpublished data). When three oncolytic HSV-1 expressing IL-12, IL-18 or soluble B7-1 were tested together using the same Neuro2a model, the triple combination of the three armed viruses exhibited the highest efficacy amongst all single virus or combinations of two viruses (25). Combining 1 x 10^5 pfu each of the three "armed" viruses showed stronger antitumor activities than any single "armed" virus at 3 x 10^5 pfu in inoculated tumors as well as non-inoculated remote tumors. We have also created a G47Δ-backbone HSV-1 double-armed with

IL-18 and soluble B7-1 (23). This double-armed oncolytic HSV-1 showed a significant enhancement of antitumor efficacy via T-cell mediated immune responses in A/J mice with subcutaneous Neuro2a tumors as well as in C57BL/6 mice bearing subcutaneous TRAMP-C2 prostate cancer.

Whereas the most common route of delivery of oncolytic HSV-1 has been a direct intratumoral inoculation, an intravenous delivery would further broaden the clinical application of oncolytic HSV-1 vectors if proven effective. One of approaches to generate therapeutic efficacy via intravenous administration is by "arming" of oncolytic HSV-1. We observed that intravenous delivery of T-mfIL12 caused a significant inhibition of tumor growth compared with mock and T-01 treatments in A/J mice bearing subcutaneous Neuro2a tumors (Guan Y, *et al.* unpublished data). Also, in a renal cancer lung metastases model using BALB/c mice and syngeneic RenCa cells, intravenous administrations of T-mfIL12 significantly inhibited the number of metastases compared with mock and T-01 treatments (Tsurumaki Y, *et al.* unpublished data).

7. SUMMARY

In summary, oncolytic HSV-1 has high potential as a new drug for cancer therapy for following reasons. 1) It can be applied to all types of solid tumor: Oncolytic HSV-1 therapy has a wide variety of application in cancer therapy. 2) It can be combined with conventional therapies, *i.e.* surgery, radiotherapy and chemotherapy. 3) It can be administered repeatedly: Neither myelosuppression nor accumulating toxicity has been observed so far. And, 4) it may work synergistically with immunotherapy.

"Arming" of oncolytic HSV-1 with transgenes leads to development of a variety of oncolytic HSV-1 with certain antitumor functions resulting in enhancement of antitumor efficacy, which in turn leads to development of a series of oncolytic HSV-1 suited for certain tumor types and different administration routes.

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Abbreviations: HSV-1: herpes simplex viruses type 1

Key Words: Oncolytic Virus Therapy, Replication-Competent Virus Vectors, Herpes Simplex Virus Type I, Antitumor Immunity, Review

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