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# Regional liver therapy using oncolytic virus to target hepatic colorectal metastases

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## Abstract

The mortality of colorectal carcinoma often results from the progression of metastatic disease, which is predominantly hepatic. Though recent advances in surgical, locoregional, and systemic therapies have yielded modest survival improvements, treatment of these aggressive lesions is limited to palliation for the vast majority of patients. Oncolytic viral therapy represents a promising novel therapeutic modality that has achieved tumor regression in several preclinical and clinical models. Evidence further suggests that locoregional viral administration may improve viral efficacy while minimizing toxicity. This study will review the theories behind hepatic arterial infusion of oncolytic virus, as well as herpes viral design, preclinical data, and clinical progress in regional liver therapy using oncolytic virus to treat hepatic colorectal carcinoma metastases.

## INTRODUCTION

Both primary and secondary hepatic malignancies are notoriously aggressive, and have long evaded traditional therapies (1;2). While recent advancements in surgical and ablative technologies hold promise, especially for patients with few or isolated lesions, hepatic metastases remain the most frequent cause of mortality resulting from colorectal carcinoma, and treatment of colorectal metastases remains primarily palliative (2–5). Additionally, despite recent chemotherapeutic improvements, many patients receiving systemic chemotherapy still suffer from the debilitating toxicities that result from low tumor specificity achieved by the vast majority of common chemotherapeutic agents (6). Regionally directed chemotherapy via hepatic arterial infusion (HAI) offers a more tumor-specific treatment modality and has been investigated in combination with systemic therapy to achieve disease responses that can facilitate tumor resection in properly selected patients, but the need for novel therapies is ever-present (7).

In recent years, oncolytic viral therapy has emerged as a promising alternative therapy for a wide range of cancers. Currently, genetically engineered attenuated viruses are the most commonly used gene delivery vehicles in clinical trials (8;9). With high tumor specificity and minimal host toxicity, viral agents hold great clinical promise. While a variety of engineered DNA and wild-type RNA viruses have been used, herpes simplex virus type 1 (HSV-1) possesses several unique characteristics that lend it superiority in both genetic manipulation and tumor selectivity (9;10). At least 12 currently published clinical trials and



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several more trials in progress have studied engineered HSV-1 subtypes with promising results against a wide range of cancers, including high-grade malignant gliomas, melanoma, squamous cell carcinomas of the head and neck, recurrent breast cancer, pancreatic cancer, and hepatic colorectal metastases (11–14). Furthermore, several authors examining engineered HSV-1 subtypes in preclinical models have found sustained viral function in both hypoxic and necrotic microenvironments typical of metastases, which are known to promote resistance to standard adjuvant therapies (15;16). These characteristics make HSV-1 an ideal weapon in the fight against hepatic colorectal metastases.

This article will review the principles of locoregional oncolytic virotherapy, the structure and function of oncolytic herpes viral constructs and their efficacy against hepatic colorectal metastases, and results of recent preclinical experiments and clinical trials using HAI of oncolytic virus.

## CONCEPT OF LOCOREGIONAL ONCOLYTIC VIROTHERAPY USING HSV-1

While survival remains dismal for patients with hepatic colorectal metastases who fail first-line systemic chemotherapy, recent studies have shown survival advantages with decreased toxicities following salvage chemotherapy administered via HAI in combination with systemic infusions (17). Furthermore, this success was achieved despite the use of doses far smaller than those used in systemic therapy. Applying these findings to viral therapy, several authors have postulated that HAI can facilitate decreased viral doses, thereby minimizing systemic toxicity and minimizing host immune system interference. Replication-competent oncolytic HSV-1 (oHSV) offers the added benefit of producing a large number of progeny from relatively few infected cells. Clinically, this translates to efficacy without the need for large systemic viral loads. HAI amplifies the already significant tumor specificity of viral therapy by allowing for the delivery of vector to the liver at concentrations far exceeding those reached systemically (9). Because these vectors are inherently oncotropic themselves, virus can be delivered at extremely high local concentrations with minimal toxicity to noncancerous tissue.

The dual hepatic blood supply also provides an anatomic advantage for HAI. Other researchers have established that whereas normal hepatocytes receive blood primarily from portal circulation, hepatic metastases receive blood supply predominantly from the hepatic artery (18). Building on this principle, experiments using HAI of chemotherapy have demonstrated that HAI delivers high drug concentrations to hepatic metastases with enhanced antitumoral effects and minimal damage to normal liver tissue (19;20). Investigators of oncolytic viral therapy took a cue from these theories and outcomes to examine viral HAI.

While viral oncolysis is often perceived as a single treatment modality, in truth it refers to a wide array of therapies based on individual virus families, each with their own unique advantages. oHSV vectors possess several unique characteristics that set them apart from other viruses currently being explored. Perhaps most importantly, HSV encodes a thymidine kinase (TK) gene that renders it susceptible to antiherpetic medications like acyclovir, which stands as a safeguard should excessive extratumoral viral replication occur (21). Furthermore, many characteristics of tumor microbiology yield a cellular microenvironment that naturally facilitates preferential viral replication compared with noncancerous tissues. For instance, rapidly proliferating tumors often outgrow their own blood supply, resulting in regions of hypoxia and tumor necrosis. Low tumor oxygen tension has been correlated with increased metastases and recurrence in a variety of cancers (22–24). Moreover, tumor hypoxia is known to facilitate resistance to standard adjuvant therapies (25;26). However, preclinical studies have demonstrated that viral replication is tolerant of hypoxia and that

oHSV can even be engineered for enhanced viral replication in a hypoxic environment (16). Still more preclinical data indicate that cancer cell death, like that found in a necrotic tumor microenvironment, can enhance HSV-1 replication in neighboring cells (15). With these principles in mind, our group hypothesized that oHSV vectors would be ideal for therapy of hepatic colorectal metastases, and tested this hypothesis in both preclinical and clinical settings (17).

## EVOLUTION OF ONCOLYTIC HERPES SIMPLEX VIRUS

Over the past two decades, breakthroughs of genetic engineering have allowed investigators to design new generations of oHSV vectors that are constantly improving in their ability to selectively infect and treat a wide range of cancers. The foundations of viral oncolysis trace back over a century, with the earliest reports of what are now known to be viral diseases inducing cancer regression prior to the discovery of viruses (27). Subsequently, interest in viral therapy ebbed and flowed, with interest in the use of natural viruses peaking in the 1950s and 1960s, marked by formal clinical trials and attempts to generate distinctly oncotropic viral strains through selective breeding (28–30). At that time, interest in viruses as potential antineoplastic therapies was abandoned due to unacceptable side effects that eventually ended the trials (31). It would not be until the emergence of modern genetic engineering in the 1990s that viral oncolysis would resurface in earnest with renewed potential as a cancer therapy. What separates the modern approach to viral cancer therapeutics from earlier experimentation is the advent of genetic engineering: the ability to manipulate the vector genome in order to augment the specificity and efficacy of treatment.

### Modern oHSV Development—The First Three Generations

While a variety of oncolytic viral vectors have yielded promising findings in experimental and clinical models, oHSV possesses several qualities that make it ideal for oncolytic therapy. It efficiently infects a broad range of cells and species, and has a large well-characterized genome, less than half of which is required for viral replication, making the virus a prime target for genetic manipulation (32). In fact, oHSV vectors can accept the largest genetic inserts (up to 30 kb) of any oncolytic virus under investigation, with the next largest adenovirus accepting only 10 kb inserts (32). Furthermore, as a replication-competent virus, oHSV can achieve replication even in quiescent cells, making it ideal for the infection of putative cancer stem cells. In terms of safeguards for the host, the virus remains inherently episomal, which lends protection from insertional mutagenesis (33), and as previously mentioned HSV-1 is the only virus under clinical investigation for which consistently effective, FDA-approved antiviral therapy is readily available (34).

The development of herpes oncolysis as a viable experimental therapy began with a finite series of strains featuring key shifts in basic vector design. These initial strains are frequently grouped into three classes or “generations” of vectors. While the field of oHSV vector design has since exploded into a wide range of diverse vectors, a brief review of these first three generations provides a convenient starting point for understanding the underpinnings of current and future oHSV design (Figure 1).

### First Generation

The first obvious requirement in the construction of a therapeutic vector is to minimize toxicity that would result from infection of noncancerous tissues. Thus, the first generation of genetically engineered oHSV vectors was created by simply deleting genes thought to be essential for infection of normal tissues but redundant for the infection of cancer cells. First generation oHSV vectors all featured mutations of genes encoding for either ribonucleotide reductase (RR), tyrosine kinase (TK), or $\gamma_1 34.5$. The common thread among these genes is

that their gene products are all crucial to viral replication in relatively quiescent normal cells, but nonessential in rapidly dividing cancer cells harboring a ready supply of viable genetic machinery. For example, the first recombinant oHSV, *dl*sptk, was generated through the deletion of the *UL23* gene encoding TK—an enzyme that processes nucleotides to facilitate replication of DNA. This mutated HSV-1 could infect individual normal cells but failed to replicate at a rate sufficient to sustain infection. Conversely, in rapidly dividing cancer cells, where a surplus of ready nucleotides is provided by an overactive native cellular replication machinery, viral replication would proceed unhindered to allow for progressive lytic infection (35).

## Second Generation

While the first generation of oHSVs offered key proofs of principle in terms of tumor specificity and decreased host toxicities, ultimately concern regarding viral resistance to first-line antiherpes medications (in the case of TK deletions) and reversion to wild type resulted in development of a new generation of multimutated vectors.

The prototypical second generation oHSV vector, G207, added a deletion of the *U39* gene (encoding RR) to the first generation 1716 strain, creating a vector that lacked both *U39* and $\gamma_1 34.5$. This G207 vector has been studied in both preclinical and clinical models, and remains in use by many labs both in its original form and as a backbone for additional manipulations. In a historical sense, the second generation of vectors is notable for first addressing all basic safety concerns required in vector design. All further developments in vector design since have focused on increasing vector efficacy rather than safety.

## Third Generation

The third generation oHSV vectors reflected recognition of the need for increased efficacy. G47Δ was created by deleting the *α47* gene and the overlapping US11 promoter region from the G207 backbone. This *α47* gene encodes for a protein that protects the virus from host immune responses by down-regulating host cell expression of major histocompatibility complex class 1 (MHC-1). Deleting *α47* thereby restores MHC-1, allowing tumor cells to present antigen to circulating T-cells in response to infection. Todo et al. showed that this deletion had the intended response of increasing tumor reduction by enhancing antitumor immune response (36). Another third generation viral construct, NV1066, deleted single copies of *α0*, *α4*, and *γ34.5*, which altogether resulted in decreased host neurovirulence with increased tumor specificity (37). NV1066 further contains an enhanced green fluorescent protein insert, which causes infected cells to fluoresce green under the proper fluorescent microscopy or laparoscopy (37).

## Arming Vectors: Insertion of Genes Encoding for Anticancer Proteins

While deletion of viral genes results in high viral tumor selectivity, incomplete tumor responses in preclinical and clinical models using these initial vectors prompted the development of oHSV strains that could augment tumor cell kill. These new vectors built on the constructs outlined above by strategically inserting genes intended to increase the vectors' anticancer efficacy without compromising the selective attenuation seen in noncancer cells. A wide variety of transgenes have been incorporated, with many different purposes. Intended functions range from activating chemotherapy prodrugs (38–43), to augmenting host immune response to tumor cells (44–49), to inducing anti-angiogenesis proteins (50–54). Still other vectors have been constructed with tumor-specific promoters, designed to respond either to certain elements of a microenvironment, like hypoxia (typical of tumor cells), or to an antigen or protein expressed predominantly by cancer cells (16;55–58). The progress of these armed vectors marks a promising future for targeted oncolytic virotherapies.

## PRECLINICAL SUCCESS

Locoregional oncolytic viral therapy has been extensively tested in a variety of cancers, with several murine models demonstrating excellent tumor response to local therapy. For example, carotid perfusion of oHSV cured experimental oral cancer in a hamster model (59), while single doses of oHSV via peritoneal and pleural perfusion in murine models have shown both tumor regression and cure in experimental animals with disseminated xenografts of human gastric cancer, colorectal cancer, esophageal cancer, and mesothelioma (60–64). Accordingly, a wealth of preclinical data have been reported by several groups showing efficacy of oncolytic viral therapy against colorectal cancer and hepatic colorectal metastases in models of both local and systemic therapy (Table 1). Kooby and colleagues were among the first to demonstrate oHSV efficacy against colorectal cancer and hepatic metastases, using G207 administered by intratumoral injection of subcutaneous tumors as well as portal infusion directed at hepatic metastases in a murine model. They noted significantly fewer nodules in the treated livers, as detailed in Table 1 (65).

While it should be mentioned that systemic therapy has also shown great success preclinically with low rates of adverse effects (66), recent evidence suggests that local viral delivery confers tumoricidal effects superior to those resulting from systemic delivery, while exposing the host organs to minimal levels of virus. For example, Kulu et al. compared intraperitoneal to intravenous administration of oHSV to treat disseminated peritoneal colorectal carcinomatosis in mice (67). After three doses, these researchers found that intraperitoneal administration resulted in more restricted biodistribution, less host toxicity, and greater efficacy against peritoneal metastases compared with intravenous administration (67). In terms of dosing frequency, preclinical studies have shown that multiple doses of both systemic and local virotherapy result in significantly enhanced tumoricidal effects, an important consideration in the development of clinical models (20;66;68).

As oncolytic viruses gained more evidence-based ground, concerns regarding host immune response were raised. Investigators wondered whether a host immune system would attack therapeutic viruses, whether viruses would prove effective against a pre-immunized host, and whether the tumor responses seen *in vivo* occurred as a result of tumor lysis, or secondary to host immune activity. Yoon et al. investigated several of these aspects by performing extensive *in vivo* testing in immune-competent and immune-deficient mice to find that oHSV-mediated tumor inhibition was equivalent, and concluded that tumor destruction is mitigated primarily by viral oncolysis rather than host immune response (69). Malhotra and colleagues further investigated the role of the immune system in viral oncolysis by comparing the effects of modified first generation oHSV subtype NV1023 to its derivative, NV1034, a subtype capable of granulocyte-macrophage colony stimulating factor (GM-CSF) secretion. Mouse flank tumors decreased in size after injection with both types of virus; however, enhanced antitumor efficacy was seen with NV1034 injection, suggesting that local cytokine production could add to the already potent antitumor effects of oHSV (70). Interestingly, Malhotra et al. also showed that intratumoral administration of both subtypes of HSV-1 protected against future tumor rechallenges, and that this protection was tumor specific (70). Armed with these and other promising preclinical conclusions, investigators proceeded to clinical trials.

## CLINICAL TRIALS

HSV-1 subtypes G207 and NV1020 have been clinically evaluated in several phase I and II trials for treatment of a variety of malignancies including malignant glioma (12;13;71;72), melanoma <12003, 18936≫, squamous cell carcinomas of the head and neck (73), recurrent breast cancer, and pancreatic cancer (74). Previous studies utilized intratumoral or

intravenous delivery of HSV-1 subtypes. Our group has utilized oHSV via an intra-arterial delivery method that demonstrates preferential viral selection for tumor tissue over normal liver tissues, as well as excellent long-term safety data (17;21).

Others have utilized alternate local treatment modalities against primary and secondary hepatic tumors, such as intratumoral virus administration. Park et al. administered a total of four doses of up to $3 \times 10^9$ plaque-forming units (PFU) of oncolytic poxvirus, JX-594, every three weeks via intratumoral injection to 14 patients with an array of primary and metastatic liver tumors (75). The authors noted radiographic disease regression in three patients and stable disease in six with side effects including fever, chills, and direct hyperbilirubinemia (75;76). With optimistic tumor responses and a similar adverse effect profile as is seen with HAI of other viral therapies, intratumoral administration affirms the safety and efficacy of locoregional oncolytic virotherapy.

Though Kemeny and colleagues were the first to use oHSV via HAI in a clinical setting, others had shown safety and efficacy of HAI using adenovirus (Table 2) (77;78). In 2001, Habib et al. described results of *dl1520* administered five times to three hepatocellular carcinoma patients intratumorally, three patients with hepatic colorectal metastases via the hepatic artery, and three additional patients with hepatic colorectal metastases intravenously in a phase I trial (78). The trial used an escalating dose design, with doses peaking at $3 \times 10^{11}$ PFU (78). Habib and colleagues further conducted a phase II trial utilizing two to four viral doses via HAI with concurrent continuous 5-fluorouracil (5-FU) in 7 patients with hepatic colorectal metastases, noting stable disease in six of seven patients and 50% carcinoembryonic antigen (CEA) reductions in three of six patients with initially elevated CEA (78). Similarly, Reid et al. established safety of HAI of Onyx-015 in nine patients with hepatic colorectal metastases (77). Toxicities were mild, but the exact nature of tumor response to virus was difficult to assess in this study, which utilized combination therapy with virus and HAI of 5-FU and leucovorin after 2 doses of Onyx-015 alone, all after varied chemotherapeutic regimens (77). In a phase II continuation of this study, with long-term follow-up, Reid et al. observed a median survival of 12 months and noted that 11 of the 24 patients (46%) examined in the phase II trial experienced either tumor reduction or tumor enlargement followed by regression of greater than or equal to 10% (79).

Similarly, our group observed favorable results using just a single dose of HSV-1 (NV1020) via HAI followed by HAI of chemotherapy in 12 patients. We noted partial responses (defined as greater than or equal to 25% tumor reduction) in all patients and a 25 month median survival, with one patient who remained alive 65 months after dosing, and 72 months after initial diagnosis (17;21). Of note, this patient was in the highest dose ($1 \times 10^8$ PFU) cohort and exhibited the most dramatic response to therapy seen in the trial (Figure 2) (17;21). In all patients, to assess the effects of virotherapy alone, NV1020 was administered without any concomitant therapy (17;21). Two of the three patients in the highest dose cohort noted respective 39% and 20% reductions in radiographic tumor volume with virus alone (17;21). With preclinical data showing significantly enhanced tumor responses with multiple doses, we expect in future trials to establish the maximum tolerated dose as a function of multiple doses in order to achieve enhanced tumor responses.

Nevertheless, the responses achieved in this study are quite remarkable when one considers that all of the patients included had previously failed 5-FU and leucovorin treatment as well as subsequent salvage regimens, consisting predominantly of irinotecan, and were exhibiting rising CEA levels at the time of viral administration (17). Furthermore, 12 patients were treated in four cohorts with each subsequent cohort receiving an incrementally increased dose. Thus, only three patients received maximum dosage of $1 \times 10^8$ PFU allowed in this study. All told, all 12 patients achieved partial responses following HAI of floxuridine with

dexamethasone one month after viral therapy, with average tumor dimensions showing sustained decreases of 35%–37% at 6–12 months, and maximum responses varying from 39%–81% tumor reductions at various time points (21). In addition to radiographic tumor diminution, all patients experienced a decrease in CEA levels. No major complications were suffered and while all patients were HSV-1 seropositive prior to the study, no patient demonstrated signs of virus reactivation (17;21). Finally, no blood, urine, vaginal swabs, or rectal swabs cultured positive for HSV (17;21). Thus, oHSV warrants further investigation as a tool against hepatic colorectal metastases.

In addition to establishment of optimum dosing and infusion schedules, future studies of HAI of oncolytic virotherapy may include evaluation of viral efficacy via correlation with CEA level. In our group's NV1020 trial, all patients experienced partial response and CEA reduction following viral and chemotherapy, and the patient with the greatest radiographic tumor reduction in response to one dose of virus alone (39%) also had the greatest reduction of CEA level (75%) (17;21). While CEA levels vary greatly between patients, relative change in CEA in a single patient has been correlated with tumor recurrence and tumor burden (80–83). Accordingly, CEA could serve as a marker for oHSV efficacy during treatment, perhaps guiding future dosing strategies in individual patients. Correlation of CEA level and tumor regression has also been confirmed in trials examining HAI of adenovirus, which further showed that radiographic response often lagged behind clinical responses like decreased CEA levels (79). With physiologic and radiologic improvements, oHSV holds substantial hope for the continued clinical success of HSV-1 subtypes against hepatic and other malignancies.

## CONCLUSION

Finally, with some authors reporting favorable extrahepatic responses with HAI in the clinical setting, it is clear that the full potential of locoregional virotherapy has yet to be realized (84). With side effects that are subjectively more tolerable than those of chemotherapy, HAI of oncolytic viruses clearly holds great promise as a successful treatment modality for patients with highly aggressive metastatic disease. This therapy warrants optimization and further testing to achieve future incorporation into first-line regimens against hepatic colorectal disease.

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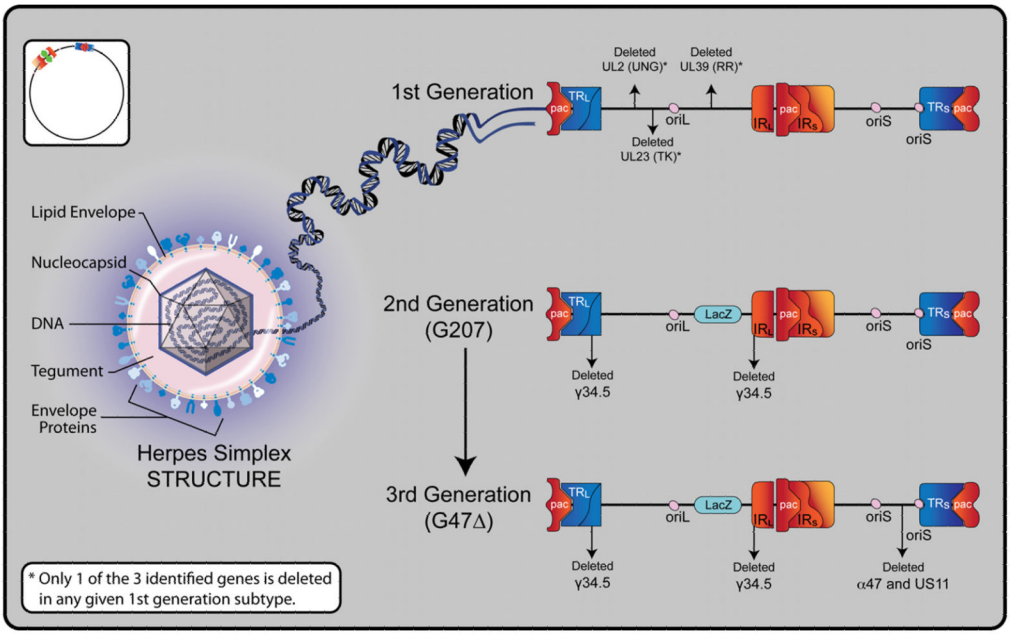
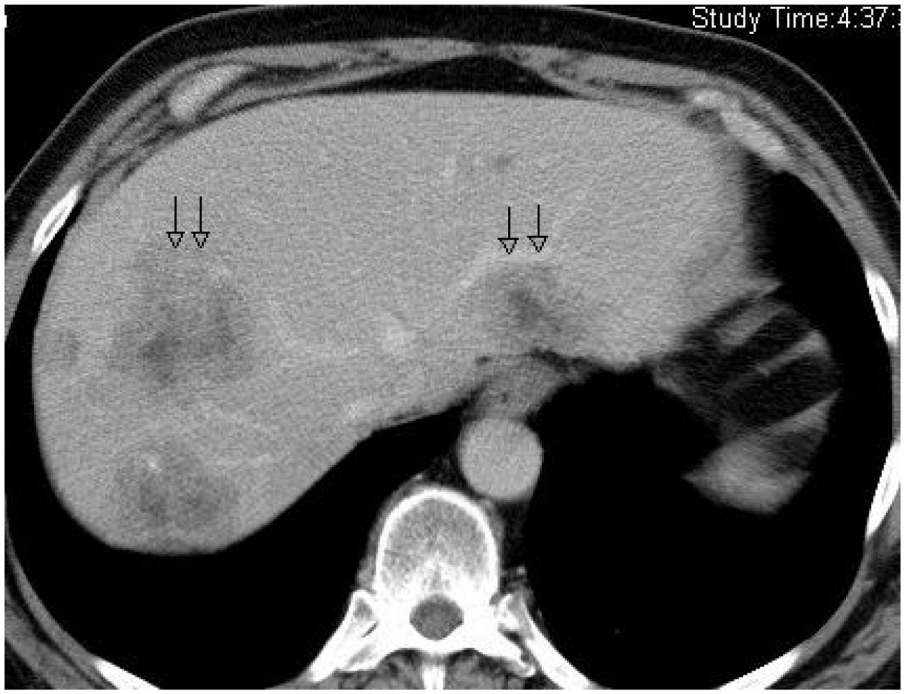



**Figure 1. Overview of Oncolytic HSV-1 Structure and Generations**

Herpes simplex virus type 1 is a double-stranded DNA virus with icosahedral symmetry. A central core contains viral DNA within a nucleocapsid. Tegument between the nucleocapsid and outer envelope contains various proteins that are transferred to a host cell upon fusion. The lipid envelope carries viral glycoproteins that facilitate cellular fusion and specificity. The 152 kb DNA genome is comprised of unique long and short segments, each flanked by inverted ($IR_L$ and $IR_S$) and terminal repeats ($TR_L$ and $TR_S$). The genome further contains three DNA packaging (pac) signals, which enable construction of virions. There are two different origins of replication, one in the unique long segment (oriL), and one in the unique short segment (oriS). Several genes are duplicated as a result of the inverted repeats. These include oriS, *γ34.5*, *α0*, and *α4*. Representative constructs are shown of each of the three generations of oncolytic HSV-1. In the first generation, potential single deletions of uracil deglycosylase (UNG), ribonucleotide reductase (RR), and thymidine kinase (TK) are represented. In the second generation, the G207 construct reflects double deletions of *γ34.5* and the addition of a *LacZ* gene at the site of *UL39* (RR), which effectively inactivates RR and enables histochemical identification of viral replication via β-galactosidase detection. In the third generation, the G47Δ construct reflects its derivation from the G207 backbone with the deletion of the *α47* gene and the overlapping *US11* promoter region. Since *α47* encodes an inhibitor of antigen presentation, its deletion prevents the down-regulation of MHC class I peptides on the surface of virally infected cells, thereby diminishing host immune responses and enhancing viral efficacy.

**2A:**




**2B:**

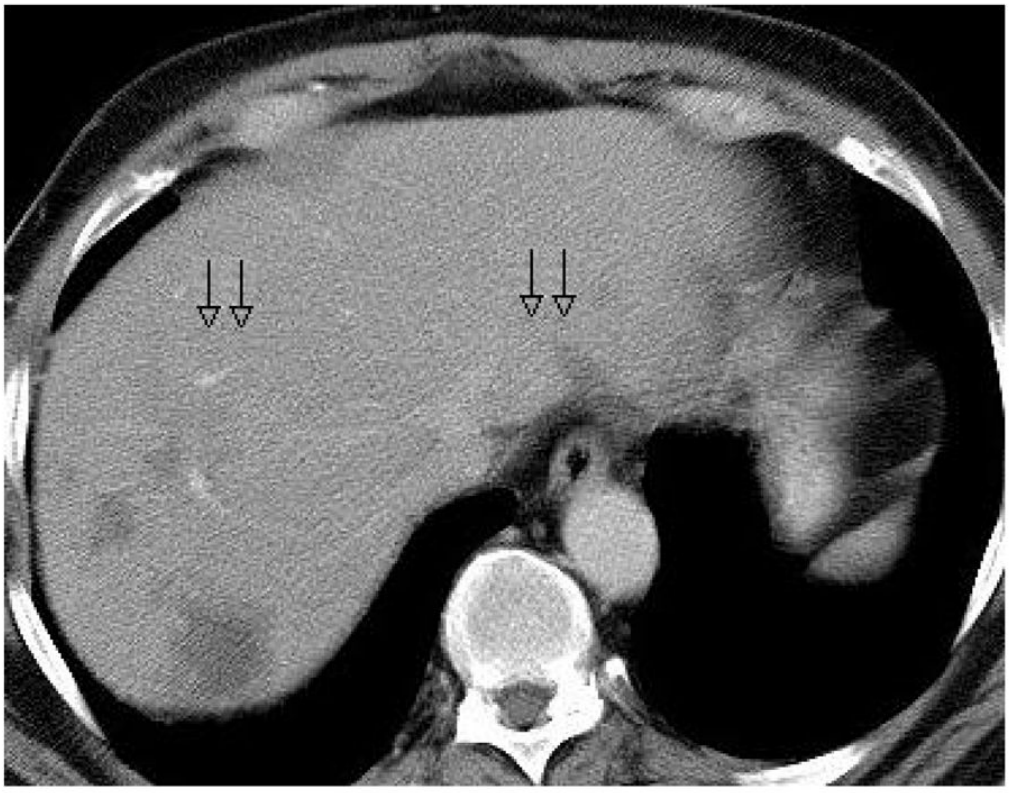


**Figure 2. Radiographic change in a single patient at maximum dose of $1 \times 10^8$ plaque-forming units**

Representative slices from computed tomography scans performed before **(a)** and one month after **(b)** oHSV treatment with NV1020 via hepatic arterial infusion. As delineated by the black arrows, the tumors have clearly decreased significantly in size with viral therapy as the only treatment modality employed between the two scans.

## Table 1

Oncolytic Herpes Simplex Virus vs. Colorectal Carcinoma and Hepatic Metastases in Preclinical Models

| Senior Author, Year | Virus | *In vivo* Model and Relevant Findings | Route/Maximum Dose |
|---|---|---|---|
| **Fong, 1999** (65) | HSV-1 (G207) | Athymic rats:<br>Flank tumors from 3 CRC cell lines—complete response, partial response, or reduced growth rate noted with viral treatment<br>Morris hepatoma model—splenic tumor challenge with portal infusion of virus 7 days later<br>11 days postinfusion, treated livers with 13 +/− 10 nodules vs. 80 +/− 30 nodules in untreated ($P<0.05$) | Flank tumors—IT $1 \times 10^7$ PFU<br>RH7777 hepatic micrometastases —portal vein $1 \times 10^8$ PFU |
| **Tanabe, 2000** (69) | HSV-1 (hrR3) | Immunocompetent and incompetent mice with diffuse liver metastases:<br>Diffuse CRC liver metastases model—splenic tumor challenge with splenic viral injection 8 days later<br>14 days postinjection, virally treated mice with 1–5 nodules vs. untreated mice with nodules “too numerous to count” | Hepatic metastases—splenic injection $5 \times 10^7$ PFU |
| **Fong, 2007** (70) | HSV-1 (NV1023 or NV1034 – GM-CSF) | Immunocompetent mice:<br>CRC and hepatoma flank tumors—viral treatment 2 weeks after tumor cell injection<br>14 days posttreatment, significantly diminished tumor volumes in virally treated groups. Enhanced efficacy of NV1034 vs. NV1023 | CT26 flank tumors—IT $5 \times 10^7$ PFU<br>Hepa 1–6 Tumors—IT, $5 \times 10^6$ |
| **Fong, 2007** (85) | HSV-1 (NV1023, NV1034-GM-CSF or NV1042-IL-12) | Immune-deficient mice:<br>CRC splenic injections—viral treatment 24 hours postinjection.<br>14 days posttreatment, all viral groups with significant reduction in mean surface liver nodules vs. control. Enhanced efficacy of NV1042>NV1034>NV1023 | CT26 splenic and hepatic micrometastases—splenic injections $1 \times 10^7$ PFU |
| **Tanabe, 2009** (67) | HSV-1 (hrR3) | Immunocompetent mice:<br>CRC peritoneal metastases—viral treatment began 4 days post CRC dissemination. Virus administered QOD for 3 doses.<br>48 hours after last viral dose, abdominal organs removed *en bloc*, tumor weight significantly lower in IP group. | MC26 peritoneal metastases—IV or IP $1 \times 10^8$ PFU |

h = hours, IT = intratumoral, PFU = plaque-forming units, CRC = murine colorectal carcinoma, IV = intravenous, IP = intraperitoneal, QOD = every other day, RH7777 = murine hepatoma cell line, CT26 = human colorectal carcinoma cell line, Hepa 1–6 = mouse hepatoma cell line, MC26 = murine colorectal carcinoma cell line.

**Table 2**

Hepatic Arterial Infusion of Oncolytic Virus vs. Hepatic Colorectal Metastases in Clinical Trials

| Senior Investigator, Year, Phase | Virus | # Pts | Dosage Range (Particles) and Schema per Patient | Toxicity Seen in >50% Patients | Tumor Response |
|---|---|---|---|---|---|
| **Reid 2001, I** (77) | Adenovirus (Onyx-015) | 11* | $2 \times 10^8 – 2 \times 10^{12}$<br>2 virus-only doses then 3 virus + 5-FU/leucovorin | Virus alone: pyrexia, chills, elevated Alk Phos & AST | Dose-response tumor reduction seen with combination therapy at $> 6 \times 10^{11}$<br>2 partial response<br>2 stable disease |
| **Reid 2002, II** (86) | Adenovirus (Onyx-015) | 27** | $2 \times 10^9 – 2 \times 10^{12}$<br>2 virus-only doses (25) then 3 virus + 5-FU/ leucovorin (18) | Virus alone: pyrexia, chills, increased Alk Phos and AST, nausea | 3 ≥ 50% regression<br>4 MR?<br>9 +/− 25% change<br>11 disease progression ≥25% growth |
| **Fong 2006, I** (17) | HSV-1 (NV1020) | 12 | $3 \times 10^6 – 1 \times 10^8$<br>Single dose—3 patients per dose cohort<br>28 days after viral dose, HAI chemo | Pyrexia, pain, diarrhea, fatigue, atelectasis, headache | After virus only: Dose-response tumor reduction<br>1 39%, 1 20% reduction<br>7 stable disease<br>3 disease progression<br>After virus + chemo: 12 partial response (range 39%–81% reduction) |
| **Habib 2001, I/II** (78) | Adenovirus (*dl*1520) | 7*** | $3 \times 10^{11}$ PFU<br>One cycle = 3 consecutive days receiving above dose. Patients received 2–4 cycles.<br>Concurrent daily HAI of 5-FU for 3 months, beginning 1 week prior to viral therapy | None (2 patients with reported “shivers,” 1 with pyrexia) | 50% CEA decrease in 3 of 6 patients with pretreatment elevated CEA<br>6 stable disease<br>1 disease progression |
| **Tawfik 2008, II** (84) | HSV-1 (NV1020) | 22 | $1 \times 10^8$ PFU<br>Up to 4 doses, 1 per week<br>2 additional cycles of chemo | Transient (<24 h) viral syndrome | Virus alone:<br>1 complete response 9 stable disease<br>Virus and chemo:<br>1 complete response, 1 partial response, 11 stable disease |

* Hepatic metastases of gastrointestinal carcinomas, 9 colorectal + 2 pancreatic.

** Hepatic metastases of gastrointestinal carcinomas, 24 colorectal + 3 pancreatic.

*** Phase I portion of study examined patients with hepatocellular carcinoma, 7 represents patients studied in phase II, all with multiple bilateral colorectal liver metastases.

Pts = patients; HSV-1 = herpes simplex virus type 1; HAI = hepatic artery infusion; Alk Phos = alkaline phosphatase; AST = aspartate transaminase, chemo = chemotherapy; CEA = carcinoembryonic antigen; 5-FU = 5-fluorouracil; MR = magnetic resonance; PFU = plaque-forming units.

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