Review article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2013, Volume 1, Issue 8
ISSN : 2321–3647(online)

Current Status and Future Perspectives of Herpes Simplex Virus as Oncolytic Virus in Cancer Chemotherapy-A Review

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ABSTRACT

Herpes simplex virus 1, by gene modification is emerging as an effective therapeutic agent to kill cancer cells. Various types of herpes simplex virus 1(HSV-1) was studied in preclinical and clinical studies. These vectors were found to be safe and effective due to their selective actions. These vectors were studied in glioma, melanoma, breast, prostate, colon, ovarian and pancreatic cancer. One of the advantage of HSV virus is the capacity to incorporate multiple transgenes within the large genome. Insertion of transgenes like antiangiogenic genes and immunostimulatory genes were found to be a positive developments in oncolytic viral therapy. Arming therapeutic genes merits further investigation for successful translation to the clinical practice. Combining oncolyticviro therapy with other modes like chemotherapy and radiotherapy has shown synergic action. This review summarizes the latest advances from preclinical results and clinical trials and the future of modified HSV1 as therapeutic mode in cancer therapy.

Key words: HSV, Antiangiogenic, Immunostimulatory, Oncolytic, Virotherapy, Radiotherapy

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Received 02 November 2013, Accepted 11 November 2013

Please cite this article in press as: Shivaprakash G. *et al.*, Current Status and Future Perspectives of Herpes Simplex Virus as Oncolytic Virus in Cancer Chemotherapy-A Review. American Journal of Pharmacy & Health Research 2013.

INTRODUCTION

Cancer is one of the leading cause of death. It is reported that 15 million new cases and 10 million new deaths are expected in 2020.[1]Currently mainstay of cancer treatment is surgery, chemotherapy and radiotherapy. Despite these modes of treatment glitches like relapse, metastasis, toxicities due to nonselective action of the treatment were not addressed. Hence there is a need for newer modality of treatment which is safer & more effective in suppressing tumor cells selectively. In this direction advances in virotherapy is a hope. Recently development in using HSV in cancer treatment isadmirable. It is a novel way to eradicate cancer cells. Many viruses are identified as oncolytic viruses.HSV has got some distinct advantages:

1.Infects broad range of cell types & species

2. Anti-viral drugs are available

3. Large genome allows the insertion of large & for multiple transgenes.

4. Host immune reactions enhance antitumor effects.[2]

5. Circulating anti-HSV-1 antibodies do not affect cell-to-cell spread of the virus[2]

6. There are HSV-1 sensitive mouse & non -human primate models for preclinical evaluation (BALB/C or A/J mice & New World owl Monkey,*A.nancymai*).[3]

7. Viral DNA is not integrated into the host genome.[2]

8. HSV combined with radiotherapies or chemotherapies produce synergistic action.[4,5]

9. Many anti herpetic drugs are available as a safeguard against unfavorable replication of virus.[6]

Types of modified HSV1 used as oncolytic viruses(Table 1)

HSV selectivity in replicating in actively dividing cancer cells is by mutations in the viral enzymes like thymidine kinase (TK). *Dlsptk*is an engineered HSV1 with the deletion of thymidine kinase gene.It showed potent antitumor activity in glioma model at high dose when it was inoculated intracranially. In cell culture, dlsptk killed two long-term human glioma lines and three short-term human glioma cell populations. In mice with implanted human gliomas, intraneoplastic inoculation of dlsptk caused growth inhibition and prolonged survival. However high dose intracranial inoculation of this vector had a risk of fatal encephalitis.[7,8]Hence other virus with reduced neurovirulence are studied. HSV1716 is derived from HSV-1(17+) strain with deletion of both copies of γ34.5 genes. It was found to be safe and effective in Glioblastoma multiforme, [9, 10] anaplastic astrocytoma[11], oral squamous cell carcinoma.[12]The safe animal data encouraged clinical trials. Three phase I trials have been completed and two phase II trials are in preparation for high grade gliomas.

Table1: Gene –modified HSV and its efficacy in preclinical and clinical studies.

Virus	Tumor-Targeting mutation	Cancer model
Dlsptk	Tk gene deletion	Malignant human glioma cells in mice model [8]
HSV1716	ΔICP34.5-/-	Glioblastoma multiforme;[9] anaplastic astrocytoma;[10,11] oral squamous cell carcinoma in phase 1 clinical trial [12]
NV1020 (R7020)	Delete 15-kb region at UL/S junction and 700bp deletion in Tk locus UL 24,UL 56	Pancreatic cancer in mice model [13]; Colon carcinoma in mice model[73]; Bladder cancer in mice model [74]; Pleural cancer in rat model [75]; Metastatic colorectal cancer in Phase I/II clinical trial[76]
G207	ΔICP34.5-/- and insert transgene lac Z	Prostate adenocarcinoma in vitro cells [77]; Glioblastoma multiforme in Phase 1b clinical trial [2,18]; Hepatocellular carcinoma in vitro cell line [78]; Colorectal cancer in rat model [79]
G47 delta	Deletion of ICP6, γ34.5,ICP47,insert lacZ	Prostate adenocarcinoma in vitro cell lines [77]; Neuroblastoma in mouse tumour model [39]; nasopharyngeal carcinoma in mice model [80]; breast cancer in mice model [81]
NV1023	UL56,ICP 47,add lacZ	Squamous cell carcinoma in murine model. [19]
DM33	ΔICP34.5 and LAT gene	Gliomas in mice [20]
HF10	Delete 3.9kbp in the right end UL and UL/IRL junction	Breast cancer in mouse model [21,82]; Malignant melanoma in mouse model[83]; Pancreatic carcinoma in Phase 1 trial.[22]
G92A	TK,US3,UL24 and insert LacZ,Alb-ICP4	Selective hepatocellular carcinoma in mice model [23]
d12.CALP	TK,US3,UL24 and insertLacZ,Cal-ICP4	Selective for soft tissue and bone tumors in mouse model [24]
GALV.fus	Transgene gibbon ape leukemia virus envelope fusogenic membrane glycoprotein	Inhibited tumor growth in mice with hepatocellular carcinoma [25]

Tk:Thymidinekinase; LacZ:E.coliLacZ; Cal:calponin promoter; Alb:albumin promoter/ enhancer

Phase I clinical trial with 12 patients of recurrent glioblastoma multiforme, intratumoral administration of SV1716 was found to be safe and effective.[10]A phase I trial has been completed for oral squamous cell carcinoma. Twenty patients with oral squamous cell carcinoma (SCC) had a single intratumoral injection of HSV1716 at a dose of 105 pfu (plaque forming unit) or 5 x 105 pfu. Injections were done at 1, 3, or 14 days before surgical resection. Intratumoral injection of HSV1716 was found to be safe.[11,12]A pilot study of intratumoral injection of HSV1716 into subcutaneous nodules of metastatic melanoma in five patients with stage 4 melanoma was conducted. Two patients each received one injection, two received two injections, and one received four injections of HSV1716. In one patient, flattening of previously palpable

tumor nodules was seen 21 days after two direct injections of HSV1716, and there was microscopic evidence of tumor necrosis in others. Immuno histochemical staining of injected nodules revealed evidence of virus replication confined to tumor cells and was found to be nontoxic.[13] A phase I/II trial is in process for malignant pleural mesothelioma.[14]A phase I trial is in progress for non-CNS pediatric cancers.[15]This vector shows considerable promise as an oncolytic virus for cancer treatment.

NV1020 and G207 are multi mutated HSV. N1020 is an attenuated HSV-1 mutant previously known as R7020.This was originally developed as vaccine but was unsuccessful .Due to safe and efficacy data in preclinical studies(Table 1), phase 1 clinical trial with 12 patients in metastatic colorectal adenocarcinoma was conducted and results showed its antitumor efficacy.[16]G207 is engineered by deletion of two copies of γ 34.5 and ICP6 gene inactivated by insertion of the E. coli LacZ gene, was shown to act by increasing cytotoxic T lymphocytes.[2]Preclinical efficacy was demonstrated in many types of cancer (Table 1).Two phase I clinical trials in glioma were completed. The results of the first trial was published simultaneously with the first trial of HSV1716 in 2000 and the results showing safety of these viruses when injected into brain tumors.[17]A Phase Ib clinical trial of G207 was recently published in which six patients with respectable, recurrent GBM were treated with two administrations of G207. Although the Phase Ib study was not designed to demonstrate therapeutic efficacy, viral replication was observed and limited evidence of anti-tumor activity was reported.[18]G47 delta is derived from HSV-1 vector G207 with an additional deletion of α47 gene. ICP47 gene normally blocks MHC class-1 mediated antigen presentation in infected cells. Deletion of ICP47 enhances the cytopathic effects and replication ability of the vector. This is responsible for the enhanced antitumor capacity of the vector compared to G207in vitro and in mice models (Table 1).

NV1023 hasdemonstrated efficiency in vitro in squamous cell carcinoma(SCC) cell lines. In SCC subcutaneous flank tumor model in immunocompetent C3H/HeJ mice, intratumoral injection with virus caused a significant reduction in tumor volume compared with saline injections. On subsequent rechallenge in the contralateral flank with SCC cells 14% of animals treated with NV1023 failed to develop tumours. The increased antitumor efficacy seen with NV1023 was abrogated by CD4(+) and CD8(+) lymphocyte depletion.[19]DM33 is a recombinant HSV-1 with deletions of ΔICP34.5 and LAT gene. It has shown efficacy against in nude mice bearing intracranial U-87 MG human gliomas. [20]

HF10 replicates more efficiently with low virulence than wild type HSV-1.It enhances the angiogenesis and induces the cytotoxic T lymphocyte response against the tumor. Clinical

studies were carried after it was found to be safe and effective in preclinical studies(Table 1). A pilot study was done in patients with metastatic breast cancer. 0.5 ml HF10 diluents were injected into test nodule, and 0.5 ml sterile saline was injected into a second nodule in each patient. Patients were monitored for local and systemic adverse effects, and the nodules were excised 14 days after viral injection for histopathological studies. The trial was tolerated well by the patients. 30-100% of the cancer cells regressed histopathologically in recurrent breast cancer. A trial for non-resectable pancreatic cancer is underway.[21]A pilot study by injecting six patients with non-respectable pancreatic cancer with three doses of HF10 was carried. All patients were monitored for 30 days for local and systemic adverse effects and were not administered any other therapeutics during this period. The result showed that virotherapy was safe and tumors response was stable disease in three patients, partial response in one patient and progressive disease in two patients.[22]This indicates the vector is a promising oncolytic virus and deserves further clinical trials in the future.

G92A was the first example of transcriptionally targeted HSV vector where ICP4 transgene was used to specifically target hepatocellular carcinomas.[23]Intrahepatic delivery of G92A did not result in liver toxicity indicating sparing of normal tissue. d12. CALP is another ICP4promoter driven vector to promote HSV replication in soft tissue and bone tumors.[24]GALV.fus is HSV-1 armed with fusogenic membrane glycoproteins. Expression of GALV.fus significantly enhanced antitumor effect of the virus. It has shown efficacy in mice hepatocellular carcinoma model.[25]

Angiostatic gene delivering HSV and its efficacy in vivo cancer models(Table 2)

Table2:Angiostatic gene delivering HSV and its efficacy in vivo cancer models.

Virus	Tumor-Targeting mutation	Cancer model
rQT3	Deletion of ΔICP34.5,ΔICP6 and insert transgene-TIMP3	Inhibited tumor growth in mice with neuroblastoma/ malignant peripheral nerve sheath tumor model[26]
AE618	Deletion of ΔICP34.5, ΔICP6 and insert transgene EAFP	Non small cell lung cancer cell lines in vitro and invivo in SCID mice [84]
bG47Δ-PF4	Deletion of ΔICP34.5,ΔICP6, ΔICP47 and insert transgene PF-4	Glioma and Malignant Peripheral Nerve Sheath Tumors in murine model [85]
bG47Δ-dnFGFR	Deletion of ΔICP34.5-/-, ΔICP6 , ΔICP47 and insert transgene dnFgf	Glioma and Malignant Peripheral Nerve Sheath Tumors in murine model [86]

TIMP3:Tissue inhibitor of metalloproteinases; EAFP: Endostatin-Angiostatin fusion protein; PF: Platelet factor; FGR:Fibroblast growth factor

HSV modified with antiangiogenic factors, can augment the antitumor activity without affecting its viral replication. rQT3 is HSV-1 armed with tissue inhibitor of metalloproteinase 3.In athymic

mice bearing human neuroblastoma or malignant peripheral nerve sheath tumor, it delayed tumor growth, increased peak level of virus and immature collagen extracellular matrix,reduced vascular density[2, 26].AE618is HSV-1 armed with therapeutic gene Endostatin-Angiostatin fusion pr.Platelet Factor 4 is a 70 amino acid secreted protein released from the alpha-granules of activated platelets. It is known to promote antiangiogenic and antitumorigenic effects. [27]PF-4 binds to heparin sulfate proteoglycans (HSPG) and chemokine receptor 3 (CXCR3) on endothelial cells to mediate its antiangiogenic effects.[28]Its overexpression has antiangiogenic and antitumor action. Binding of fibroblast growth factor (FGF) to its receptors leads to receptor dimerization and autophosphorylation, and initiates a cascade of signal transduction events which regulate diverse processes such as apoptosis, proliferation, migration, and angiogenesis.[29] Normally, fibroblast growth factor receptor (FGFR) signaling is highly deregulated in multiple cancers including breast, prostate, thyroid, melanoma and MPNSTs(Malignant Peripheral Nerve Sheath Tumor)(30,31,32).Blockade of FGF signaling in tumors has shown promise in several animal modelsasan antiangiogenic and anti-cancer therapeutic approach (Table2).bG47Δ-PF4 and bG47Δ-dnFGFR both have the ability to inhibit the tumor growth and angiogenesis in both U87MG glioma and mouse malignant peripheral nerve sheath tumor models(Table 2).

Modification of HSV1 with immunostimulatory genes (Table 3)

In immunocompetent animals HSV-1 vectors was presumed to act by direct cytotoxic activity and indirectly by induction of antitumor immune response.[3]The antitumor immune response included a CD8+ T- cell component generated against specific tumor cell antigens.[33]. Many recombinant HSV vectors have been developed with the goal ofexploiting the immune milieu either to retract viral - induced MHC class I down- regulation (by deletion of ICP47) or to deliver regulatory cytokines.

Table3: HSV1 with immunostimulatory genes and its efficacy in vivo cancer models.

Virus	Tumor-Targeting mutation	Cancer model
HSVM002	Deletion of ΔICP34.5 insert IL-12α/IL-12β	Glioma and neuroblastoma in mice [34]
NV 1042	15Kb deletion of UL56-ICP4 promotor region and insert transgene IL-12 α/IL-12β	Glioma and neuroblastoma in mice[19]; Squamous cell carcinoma of colorectal cancer in murine model;[19] Hepatic carcinoma in male buffalo rats;[87] Prostate cancer in mice.[88]
R8306	ΔICP34.5 add transgene IL-4	Glioma in mice [35]
rHSVQ1-mIL4	ΔICP34.5 and ΔICP6 add transgene IL-4	Glioma in mice [36]

NV1034	15Kb deletion of UL56-ICP4 promotor region and insert transgene GM-CSF	Murine Squamous cell carcinoma,[19] metastatic colorectal liver murine model [37]
rHSVQ1-mCD40L	34.5 both copies deleted and insert CD40L	Metastatic brain tumor model [36]
rHSVQ1-m6CK	34.5 both copies deleted and insert 6CK	Mouse glioma model [36]
T-mfIL12	triple deletions in the *34.5*,*ICP6* and *47* genes add transgene mouse fusion type IL-12	Neuro2a tumour in mice Renal Cell Lung Metastasis model [2] Prolonged survival
T-01	triple deletions in the *34.5*,*ICP6* and α*47* genes	when A/J mice bearing intracerebral Neuro2a tumors [38]
G47ΔIL-18/B7	Deletion of ΔICP34.5,ΔICP6 and ICP 47 and insert transgene IL-18;B7-1	Neuro2a tumour and prostate cancer in mice [39]
vHsv-B7.1-Ig	Deletion of ΔICP34.5, ΔICP6 and insert transgene B7-1	Neuroblastomain mice model [40]
Oncovex GM-CSF	Deletion of ΔICP 34.5 and viral ICP 47genes;Insertion of GM-CSF	Breast cancer; head and neck cancer; gastrointestinal cancers; malignant melanoma, All in phase 1 trial [47]
HSV1-TNFα	Deletion of ΔICP34.5, α47 gene and insert TNFα	A20 double flank tumour model in Balb/C mice and Fadutumour model in nude mice [48]

CK:Chemokine; GM-CSF:Granulocyte macrophage colony stimulating factor

HSVM002 vector is derived from mutant R3659 by deleting γ34.5inserting transgene IL-12 α/IL-12β and NV1042 is derivative of NV1020 obtained by with insertions E.coli LacZ gene within ICP47 and inserting transgene IL-12 α/IL-12β.Both these IL12 expressing vectors were found to be effective at inhibiting tumors in brain(Table 3). Immunohistochemical analysis revealed significant influx of CD4+,CD8+ T cells and macrophages.[34]R8306vector wasa HSV recombinant virus in which both copies of the g34.5 gene were replaced with the murine genes encoding the cytokine interleukin-4 (IL- immunohis4). The results in vitro showed HSV-1 expressing IL-4 genes was able to infect and destroy glioma cells.In vivo experiment in mice, the IL-4 mutant prolonged median survival and was associated with striking infiltration of the tumor by macrophages, CD4 + and CD8+Tcells. [35].r HSVQ1-mIL4-The expression of IL4 was confirmed to enhance the HSV-mediated antitumor effects with the metastatic mice brain tumor

model. [36]NV1034 is another recombinant vector and derivative of NV1020 with insertions E.coli LacZ gene within ICP47 and GM-CSF.In mice with squamous cell carcinoma its efficacy in inhibiting the tumor growth was lesser compared to NV1042.[19]In a murine metastatic colorectal liver model, this virus reduced metastases with greater efficacy compared to control virus NV1023.[37]rHSVQ1-mCD40L and rHSVQ1-m6CK were created from G207-like second generation oncolytic HSV-1 armed with CD-40 ligand a 6CK respectively.The expression of CD40L and 6CK was confirmed to enhance the HSV-mediated antitumor effects with the metastatic brain tumor model.[36]

T-01 and T-mfIL12:T-01 is a new oncolytic HSV-1. T-01 has triple deletions in gamma *34.5*, *ICP6* and alpha*47* genes, which confers high replication capability and cytotoxic activity selective to cancer cells without compromising the safety(Table 3).[38]T-mfIL12 is created by deleting 34.5, α47 genes and inserting IL12 in place of ICP6. When A/J mice bearing intracerebral Neuro2a tumors were treated by a single stereotactic inoculation, both T-01- and T-mfIL12-treated animals showed a significantly longer survival than mock-treated animals. T-mfIL-12 also showed strong cytopathic activity against murine neuroblastoma comparable to T-01. When A/J mice bearing neuro2a subcutaneous tumors in bilateral flanks were treated by intratumoral inoculations into the left tumor only, T-mfIL12 showed a significantly better antitumor activity than T-01 not only in the inoculated left tumors but also in the non-inoculated remote tumors.[2]

G47ΔIL-18/B7 was created with G47ΔHSV-1 backbone inserting transgene IL-18 and B7-1**.**In vitro, recombinant vectors exhibited cytopathic effects similar to the parental G47Delta. In two immune competent mouse tumor models, prostate cancer and neuro2a neuroblastoma, the vector expressing both mIL-18 and B7-1-Ig showed a significant enhancement of antitumor efficacy via T-cell-mediated immune responses.[39]vHsv-B7.1-Igusing bacterial artificial chromosome and two recombinase-mediated recombination's, *Ino Y et al* simultaneously created four "armed" oncolytic HSV-1, designated vHsv-B7.1-Ig, vHsv-interleukin (IL)-12, vHsv-IL-18, and vHsv-null, which express murine soluble B7.1 (B7.1-Ig), murine IL-12, murine IL-18, and no transgene, respectively. These vectors possess deletions in the gamma34.5 genes and contain the green fluorescent protein gene as a histochemical marker and the immunostimulatory transgene inserted in the deleted ICP6 locus. The triple combination of vHsv-B7.1-Ig, vHsv-IL-12, and vHsv-IL-18 exhibited the highest efficacy among all single vHsv or combinations of two viruses in efficacy test in A/J mice harboring s.c. tumors of syngeneic and poorly immunogenic Neuro2a neuroblastoma.[40]

Oncovex GM-CSF is a genetically engineered strain of HSV-1 with deletion of two copies of ICP34.5 genes and ICP47genes and insertion of transgene GM-CSF.Its antitumor effect is by direct lytic effect and by immune response. OncovexGM-CSF exhibited therapeutic response via increasing antigen-specific T cells response and decreasing the level of suppressor CD4+(regulatory T cells), CD8+(suppressor T cells) and myeloid-derived suppressive cells (MDSC).[41]50 patients with advanced melanoma (most of whom had failed previous treatment) were treated with JS1/34.5-/47-/granulocyte-macrophage colony-stimulating factor (GM-CSF). The overall response rate (patients with a complete or partial response as per RECIST criteria) was 26% (16% complete responses, 10% partial responses). Another 4% of patients had a surgical complete response, and another 20% had stable disease for at least 3 months.[42]

Phase III randomized, open-label trial compared talimogenelaherparepvecwith subcutaneously administered GM-CSF (2:1 randomization) in 430 patients with unresectablestage IIIB, IIIC or IV melanoma. The primary endpoint was durable response rate (DRR), defined as a complete or partial tumor response lasting at least 6 months and starting within 12 months of treatment. DRR was achieved in 16% of patients receiving talimogenelaherparepvec compared with only 2% in the GM-CSF control group ($P<.0001$). The greatest benefit was seen in patient with stage IIIB or IIIC melanoma, with a 33% DRR vs 0% with GM-CSF. 11% of patients experiencing a complete response.[43]Phase 1b/2, multicenter, open-label trial to evaluate the safety and efficacy of talimogenelaherparepvec and ipilimumab compared to ipilimumab alone in subjects with previously untreated, unresectable, stage IIIb-IV melanoma.[44]Phase II trial with 17 patients with stage III or IVA squamous cell carcinoma head and neck (SCCHN) received one of 4 different doses of HSV GM-CSF along with concomitant cisplatin and radiotherapy. 93% of patients had a pathological complete response confirmed by neck resection. 82% of patients had a complete or partial radiologic response as per RECIST criteria. The remaining patients had stable disease, with no patient (0%) experiencing disease progression. 77% of patients remained relapse-free as of 2010.[45]Phase I studies in other cancers was done like inpancreatic, breast, and colorectal cancers. OncoVEXGM-CSF is well tolerated and can be safely administered using the multidosing and results concluded an antitumor effect.[46, 47]

The in vivo efficacy and toxicity of HSV1 expressing TNFα were compared using A20 double flank tumour model in Balb/C mice and Fadutumour model in nude mice. The results demonstrated that TNFalpha expression showed improved anti-tumour effects and without the toxic side effects.[48]

Combination therapy of HSV vectors with conventional therapy (Table 4)

Radiotherapy or chemotherapy increases the expression of GADD34.The function of GADD family protein is to stop cell cycle progression at G1 and G2 checkpoint for viral DNA repair. Up-regulation of GADD34 increases viral protein synthesis and viral replication and increased antitumor activity. Up-regulation of GADD34 expression by radiation in tumor cell infected with virus was seen in head- and-neck cancer,cholangiocarcinoma and lung cancer.[49]

G207 virus in cisplatin- sensitive human head and neck squamous cell carcinoma in an animal model combined G207 and cisplatin was more effective (100% cure) than either cisplatin (14% cure) or G207 alone (42% cure).[50] HSV virus 1716 in combination with mitomycin C (MMC) effects was examined in a murine xenograft model(NCI-H460 flank tumors). HSV-1716 (4 x 106 PFU) was injected directly followed by intravenous mitomycin C (MMC) administration (0.17 mg/kg) 24 hr later. After 3 weeks, the mean tumor weight in the combined treatment group was significantly less than either individual treatment in an additive manner.[51] NV1020(R7020) demonstrated enhanced tumor lytic effect in experimental hepatoma model. Ionizing radiation significantly enhanced the ability of R7020 to replicate in hepatoma cell lines (Hep3B) xenografts up to 14 days after infection and irradiation.

Table4: Combination of HSV vectors with conventional therapy in different cancers.

Virus	Combined with	In vivo Cancer model	Result
G207	Cisplatin	Squamous cell carcinoma in athymic mice	100% cure[50]
1716	Mitomycin C	Lung cancer model	Additive[51]
NV1020 (R7020)	Radiation	Glioma and Hepatoma model in mice	Enhanced lytic activity of virus[52,53]
rRp450	CPA and GCV	Athymic mice model	Efficacy seen [54,55,56]
MGH2	CPA and irinotecan	Mice glioma	Increased antitumor activity[57]
HSV1yCD	5-FC	BALB/c mice with diffuse liver metastasis of colon cancer	Antitumor activity was more when HSV1yCD combined with 5-FC[58]
OncoVEX^{GA} LV/CD	5-FC	Rat glioma model	More effective than control virus[59]
R3616	Gemcitabine	Mouse model of pancreatic cancer	Synergistic in advanced pancreatic cancer[61]
FusOn-H2	CPA	Mice model	lung carcinoma[63]

GCV:Ganciclovir; 5-FC: 5-Fluorouracil; CPA:Cyclophosphamide;CD:Yeast cytosine deaminase;

There was 2.5-fold increase in viral yield in irradiated Hep3B xenografts compared with non-irradiated xenografts. Intratumoral injection of R7020 into Hep3B xenografts was effective for xenografts 260 mm 3, R7020 alone was not as effective for larger tumor xenografts. Hep3B xenografts treated with ionizing radiation alone also demonstrated effective initial response rates, but regrew. In contrast, none of the Hep3B xenografts treated with both R7020 and ionizing radiation regrew during the duration of the experiment.[52]It has also shown efficacy in glioma model in mice.[53]

Herpes simplex viral mutantrRp450replicates in and kills tumor cells selectively. Created by deletion of UL39, the gene encoding for ICP6.This peptideprovides ribonucleotidereductase (RR3) activity, which is essential for viral replication and lysis of quiescent cells.An additional genetic modification was engineered into rRp450's genome by inserting the CYP2B1 gene, encoding the enzyme responsible for activating the prodrug cyclophosphamide (CPA) into its anticancer metabolite, phosphoramide mustard. Naturally, rRp450 also possesses its own endogenous HSV-TK gene, encoding the enzyme responsible for activating the prodrug GCV into its anticancer metabolite.It expresses, in infected cells, the cyclophosphamide (CPA)-sensitive rat cytochrome P450 2B1 (CYP2B1) and the ganciclovir (GCV)-sensitive herpes simplex virus thymidine kinase (HSV-TK) transgenes. It was observed rRp450 treatment of tumors in vitro and in vivo provides multimodal treatment through viral oncolysis and the two synergistic gene therapies.The combination of rRp450 oncolysis and its two synergistic gene therapies produced regression of established tumors in animals. rRp450 alone or in combination with GCV, CPA, or GCV plus CPA significantly inhibited tumor growth when compared to the saline or CPA alone controls.It was tested in rat and human glioma cell lines in vitro and in vivo after injecting 9L tumor cellssubcutaneously into theflanks of athymic mice and were then treated with rRp450 and/or prodrug(s) (Table 4).[54,55,56]

MGH2 is aengineered mutant herpes simplex virus type 1 with deletions in the viralUL39 and g134.5 genes and an insertion of the two prodrug activating genes, CYP2B1and secreted human intestinal carboxyl esterase which converts inactiveprodrugs, cyclophosphamide and irinotecan (CPT-11)intotheir active metabolites respectively. This new oncolytic virus(MGH2) displays increased antitumor efficacy against humanglioma cells both in vitroandin vivowhen combined withcyclophosphamide and CPT-11 without affecting viral replication. Therefore, MGH2 showed effective multimodal therapyfor gliomas in preclinical models when combined with thesechemotherapeutic agents.[57] Other viruses which were found to be effective when combined with anticancer agents are HSV1yCD(HSV1yeast cytosine deaminase)combined with 5

fluorouracil. Intratumoral viral replication combined with 5-fluorouracil (5-FC)bio activation significantly reduces liver tumor burden and prolongs survival in mice.BALB/c mice bearing diffuse liver metastases were treated with asingle portal venous injection of $5x10^7$ pfu HSV1yCD or media. Livers of mice in the control group contained numerous (greater than 50) tumor nodules, whereas livers of mice treated with HSV1yCD contained fewer than five.Median survival of mice treated with HSV1yCD and 5-FC was alsosignificantly greater than that of mice that received only HSV1yCD or5-FC and was three times that of untreated controls. The study concluded that intratumoral generation of 5-FU enhances the anti-neoplastic effects of HSV-1-mediated oncolysis of diffuse livermetastases.[58]

OncoVEX$^{GALV/CD}$ virus is modified by deletion of ICP34.5 and ICP47 and combined expression of a highly potent prodrug activating gene [yeast cytosine deaminase/uracil phospho-ribosyltransferase fusion (Fcy:Fur)] and the fusogenic glycoprotein from gibbon ape leukemia virus (GALV). In rats bearing subcutaneous glioma, OncoVEX $^{GALV/CD}$ combined with systemic 5-FC administration was found to be more effective than control viruses.[59] R3616 is another HSV modified by deletion of two copies of γ34.5genes.This induces T cells, macrophages and dendritic cells in murine colon cancer model.[60] Combination with gemcitabine produces synergistic action in advanced pancreatic cancer.[61]FusOn-H2 is HSV with N-terminus of the HSV-2 ICP10 gene product containing a well-defined serine/threonine protein kinase (PK) domain, which can activate the Ras/MEK/MAPK mitogenic pathway and thus facilitate efficient HSV-2 replication. Infection of FusOn-H2 led to syncytia formation in tumor cells, providing an additional tumor-destroying mechanism.[62]It causes apoptosis by targeting Ras signaling pathway. Co-administration with cyclophosphamide has shown synergistic interaction against lung carcinoma in mice.[63]It has shown potent oncolytic activity against renal cell carcinoma[64], metastatic ovarian cancer[65], pancreatic cancer[66] and human breast cancer[67].

Challenges ahead

Research to explore the interaction of HSV with immunity has to be done since many of the animal studies were carried in immune cell depleted models. Studies to explore the mechanism of how mutant viruses escapes from body immunity and from pre-existing antibodies is interesting as some animal studies indicate pre-existing antibodies do not interfere with its efficacy.[68, 69,70,71]

HSV is known to stay for long in dormant state in ganglia. Long term safety assessment studies is required with these vectors.

Though synergistic interaction is observed with conventional chemotherapy and radiotherapy exact mechanism still needs to be explored. This will enhance the scope of using virus with various chemotherapeutic regimen and reduce the toxicity of conventional modes of cancer therapy. This will also explain how the virus replication is less affected with radiotherapy and chemotherapy.

Future research in the area of cell carriers, chemical coating, virus premixed with complement inhibiting agents- dextran sulfate, altering tropism by modifying viral receptors may enhance its efficacy and safety. Newer strategies of using HSV1 mutants like in-vivo imaging or monitoring of viral infection and replication [2] and using stem cells to deliver oncolytic virus are being experimented currently.[72]

CONCLUSION

Despite the developments in using modified HSV as oncolytic agent, we are still in the preliminary stage considering its translation from laboratory to clinical use. Different generation of genetically engineered HSV1, modified by altering the genes responsible for enzymes, angiostatic genes, immunostimulatory genes has shown its potential to be a safe and efficient oncolytic virus for the treatment of cancer. Currently very few vectors were tried in clinical trials. Combined therapeutic regimen is a promising strategy to treat the cancer effectively at lesser toxicity. Still lot of research is required to use HSV as a standard therapy in cancer treatment though information till date points to be positive.

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