Oncolytic Virotherapy

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REVIEW

Oncolytic herpes viruses, chemotherapeutics, and other cancer drugs

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Abstract: Oncolytic viruses are emerging as a potential new way of treating cancers. They are selectively replication-competent viruses that propagate only in actively dividing tumor cells but not in normal cells and, as a result, destroy the tumor cells by consequence of lytic infection. At least six different oncolytic herpes simplex viruses (oHSVs) have undergone clinical trials worldwide to date, and they have demonstrated an excellent safety profile and intimations of efficacy. The first pivotal Phase III trial with an oHSV, talimogene laherparepvec (T-Vec [OncoVex^{GM-CSF}]), is almost complete, with extremely positive early results reported. Intuitively, therapeutically beneficial interactions between oHSV and chemotherapeutic and targeted therapeutic drugs would be limited as the virus requires actively dividing cells for maximum replication efficiency and most anticancer agents are cytotoxic or cytostatic. However, combinations of such agents display a range of responses, with antagonistic, additive, or, perhaps most surprisingly, synergistic enhancement of antitumor activity. When synergistic interactions in cancer cell killing are observed, chemotherapy dose reductions that achieve the same overall efficacy may be possible, resulting in a valuable reduction of adverse side effects. Therefore, the combination of an oHSV with "standard-of-care" drugs makes a logical and reasonable approach to improved therapy, and the addition of a targeted oncolytic therapy with "standard-of-care" drugs merits further investigation, both preclinically and in the clinic. Numerous publications report such studies of oncolytic HSV in combination with other drugs, and we review their findings here. Viral interactions with cellular hosts are complex and frequently involve intracellular signaling networks, thus creating diverse opportunities for synergistic or additive combinations with many anticancer drugs. We discuss potential mechanisms that may lead to synergistic interactions.

Keywords: combination studies, herpes simplex virus, oncolytic virus, virotherapy

Introduction

Using viruses to treat cancer is not a new idea. For more than 100 years there have been clinical observations that cancer patients who contracted viral infections would enter periods of remission.[1] During the 1950s and 1960s, there was considerable activity using wild-type viruses as anticancer treatments, but many of these trials were limited by the toxicity of the wild-type virus (for a historical perspective see Kelly and Russell[1]). Progress has only recently been possible as advances in virology and molecular biology have allowed either the identification of naturally occurring viruses with intrinsic tumor selectivity or by genetically engineering oncolytic viruses.

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Oncolytic herpes simplex virus (oHSV)

Oncolytic herpes viruses are attenuated, replication competent, herpes simplex type 1 viruses that selectively infect, replicate within, and lyse cancer cells. One of the first reports of an oncolytic virus being used for cancer therapy was in the early 1990s when Martuza et al[2] showed that a replication competent thymidine kinase negative herpes simplex virus (HSV)-1 mutant effectively prolonged survival of nude mice bearing intracranial glioma. Since then, numerous oHSVs have been described, most of which have deletions in either *RL1*, *UL39*, or both.

ICP34.5, the protein product of the *γ34.5* gene, is a specific determinant of neurovirulence. It plays a key role by facilitating escape from a major host defense mechanism involving the protein kinase R-mediated innate immune response pathway by directly interacting with protein phosphatase 1α to dephosphorylate eIF2α (Figure 1).

In contrast, oncolytic HSV, which lacks functional ICP34.5 protein, cannot dephosphorylate eIF2α. Thus, infection with an ICP34.5 null virus causes the host cell to shut down protein synthesis, hence, preventing the virus from replicating in normal cells. Cancer cells, however, in the course of transforming to malignant cells have impaired antiviral mechanisms that permit unimpeded viral replication.[3]

UL39 is the HSV gene encoding for the large subunit of ribonucleotide reductase (RR), the main rate limiting enzyme for viral DNA synthesis and replication, controlling the nucleotide substrate pool by regulating the conversion of ribonucleotides to deoxyribonucleotides. HSV RR is required for growth in nondividing cells but not in rapidly dividing cells, in which there is ample cellular RR for the virus to utilize. Oncolytic HSV with a defective *UL39* gene exclusively replicates in and lyses rapidly dividing cancer cells, as such cells provide sufficient levels of RR activity[4] (for comprehensive review of oHSV see Cassady and Parker,[5] Manservigi et al,[6] and Varghese and Rabkin[7]).

Modified (armed and targeted) oHSV

The concept of using viral vectors to deliver therapeutic genes to tumors is well established. Many studies have evaluated both the oncolytic and antitumor activity, and the antitumor immune response of oncolytic viruses engineered to express either immunostimulatory genes or therapeutic genes, including those that can activate prodrugs.

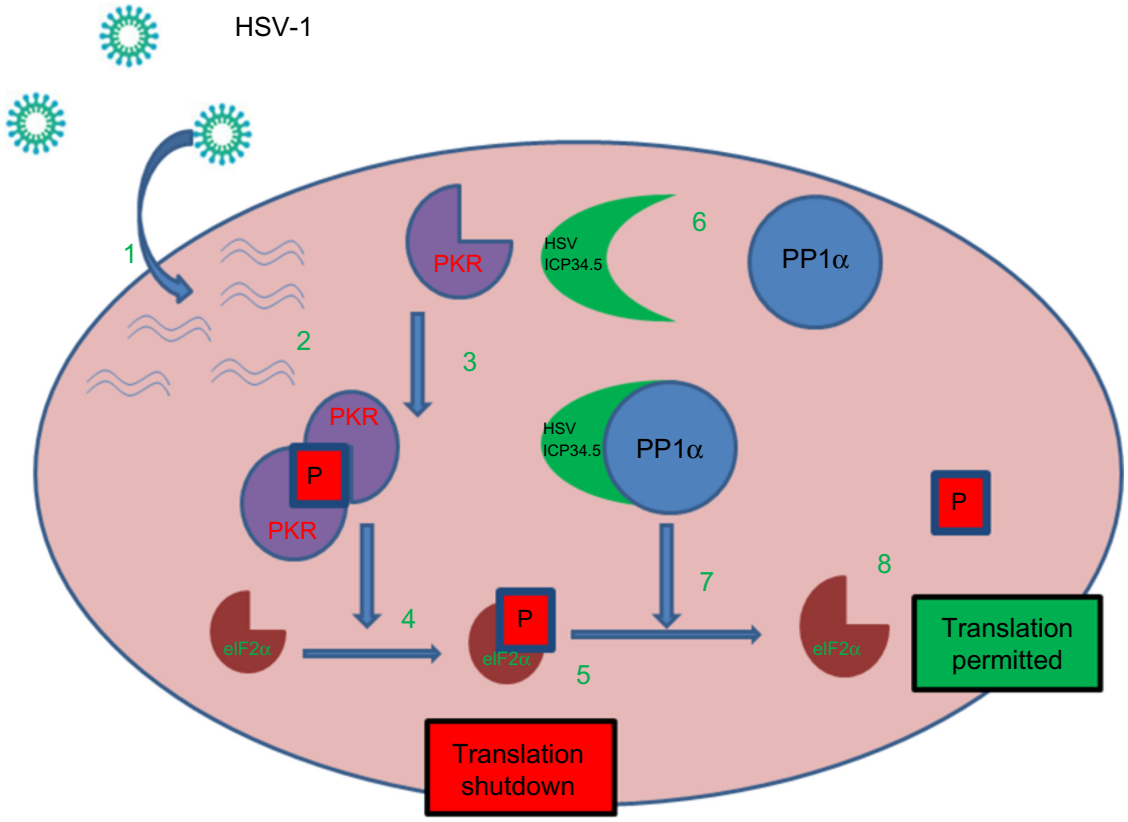

Figure 1 HSV-1 can overcome normal cells protective block in protein synthesis: 1. HSV-1 enters the host cell and begins replication. 2. Complementary RNA anneal to produce dsRNA. 3. PKR binds dsRNA, dimerizes resulting in activation and autophosphorylation. 4. Phosphorylated PKR selectively phosphorylates eIF2α. 5. Phosphorylated eIF2α causes the host cell to shutdown translation thereby preventing viral replication. 6. HSV produced ICP34.5 which forms a protein complex with PP1α. 7. The ICP34.5 PP1α complex dephosphorylates eIF2α so the viral replication (8) can continue unchecked.

Abbreviations: HSV, herpes simplex virus; PKR, protein kinase R; eIF2α, eukaryotic initiation factor 2; PP1α, protein phosphatase 1 alpha; ICP, infected cell polypepetide; P, phosphorylation.

The therapeutic efficacy of oncolytic HSV vectors encompasses two modes of action: direct oncolysis by the virus itself and indirect induction of an antitumor response. By arming viruses with genes that encode for immunomodulatory proteins such as IL(interleukin)-12,[8-10] IL-2,[11] soluble B7.1-Ig,[12] or granulocyte macrophage colony-stimulating factor (GM-CSF)[13–16] to help promote the antitumor immune response, the modified viruses are more efficacious.

Virus-directed enzyme prodrug therapy systems have also been utilized with oncolytic HSV. There are numerous reports of viruses that have been modified to code for enzymes that catalyze prodrugs into active substrates, such as HSV1yCD, a modified HSV coding for the yeast cytosine deaminase (CD) enzyme. HSV1yCD converts the nontoxic 5-fluorocytosine into fluorouracil (5-FU), a highly toxic chemotherapeutic agent,[17] rRp450 carrying rat cytochrome P450 (CYP2B1) (which converts cyclophosphamide into the alkylating toxin phosphoramide mustard),[18] and nitroreductase, which converts the prodrug CB1954 to an active alkylating agent.[19] The extensive field of oncolytic HSV vectors modified for enhanced efficacy is beyond the scope of this review; the major approaches are detailed here but reviewed in greater detail by Varghese and Rabkin.[7]

Table 1 lists the principal oHSV in clinical development. At least six different oHSV have undergone clinical trials worldwide to date. oHSV have demonstrated excellent safety profiles and, in numerous studies, signals of efficacy. The first Phase III trial with an oHSV, talimogene laherparepvec (T-Vec [OncoVex^{GM-CSF}]) has almost been completed. Initial extremely encouraging findings of the trial have been reported, with T-Vec demonstrating a statistically significant improvement in durable response rate.[20]

Oncolytic viruses in combination with chemotherapy

The use of many chemotherapeutic agents is limited by severe dose limiting toxicities and the emergence of resistant disease.[21] In comparison, the mode of action of oncolytic viruses (lytic infection) means that cancer cells are unlikely to become resistant to them. Furthermore, oncolytic viruses have a high therapeutic index (ie, the comparison of the amount of a therapeutic agent that causes the therapeutic

Table 1 Oncolytic HSVs in clinical trials

HSV strain	Genetic modification	Stage/clinical indication	Results	References
OncoVex GM-CSF (T-Vec)	Deletion in both copies of ICP34.5 + ICP47 disruption	Phase I/II and III melanoma	Evidence of virus replication in injected and adjacent uninjected tumors (head and neck). Regression of injected and uninjected tumors in late stage melanoma.	13,20,97,98
		Head and neck cancer		
		Advanced metastatic melanoma		
			Ongoing	71
R7020 (NV1020)	Deletion of one copy of ICP34.5 + tk under ICP4 promoter control + deletion in UL24, 55, and 56	Phase I and II colorectal cancer liver metastases	In Phase II disease stabilization in 40%–45% of cases.	99–102
G207	Deletion in both copies of ICP34.5 + disruption of UL39	Phase I, IB, and II recurrent brain cancer (glioma, astrocytoma, and glioblastomas)	Well tolerated. Evidence of viral replication and radiographic and neuropathological signs of antitumor activity.	103–109
HSV1716	Deletion in both copies of ICP34.5	Glioma Phase I Melanoma HNSCC	Well tolerated, no toxicity. In Phase I/II (recurrent glioblastomas) three out of 12 patients showed disease stabilization. No toxicity in melanoma or HNSCC. Evidence of viral replication in tumors.	110–114,129,130
		Non-CNS solid tumors	Ongoing Phase I	
		Malignant pleural mesothelioma	Ongoing Phase I/IIa	
HF10	Spontaneous generation of HSV-1 variant	Pancreatic cancer Recurrent breast cancer Bladder cancer HNSCC		115–122
rRp-450	ICP6 deleted and expresses prodrug enzyme for cyclophosphamide (ratCYP2B1)	Phase I liver metastases and primary liver tumors	Ongoing	131

Abbreviations: HSV, herpes simplex virus; ICP, infected cell polypepetide; tk, thymidine kinase; UL, unique long; HNSCC, head and neck squamous cell carcinoma; CNS, central nervous system.

effect, to the amount that causes toxicity) with very limited toxicities. Table 2 summarizes the potential advantages of oncolytic virotherapy.

Viral infection initiates many complex host defense pathways;[22] however, viruses have coevolved equally complex countermeasures to circumvent these activities.[23,24] Many of these countermeasures are retained by their oncolytic variants (Table 3 outlines the main cellular and viral pathways activated upon viral infection). As chemotherapeutic and targeted anticancer agents target key cellular processes that also involve complex intracellular signaling networks, there are extensive opportunities for antagonistic and synergistic interactions with oncolytic viruses, and these need to be explored and understood as the clinical acceptance of oncolytic HSV looks increasingly likely.[25]

Combining these two very different modalities in order to increase cancer cell killing is a rational approach. The clinical implications of this combination therapy are not limited to enhanced efficacy. The dose reduction index, the most relevant clinical parameter derived by Chou and Talalay analysis,[26] reveals the potential for significant dose reductions without compromising tumor cell kill. Reducing the dose of drugs such as chemotherapeutics would minimize the toxicity and may allow patients to remain on an otherwise intolerable regime, or increase their quality of life whilst still receiving treatment for their disease.

Since the initial groundbreaking studies by Toyoizumi et al[27] with HSV1716 and four standard chemotherapeutic drugs, methotrexate, cisplatin, mitomycin C, and doxorubicin, there have been many reports of the increased efficacy of oHSV in combination with a wide range of existing and potentially new anticancer drugs. Tables 4–8 present the wide variety of different combinations that have been examined, and also summarize the results. The aim of this review is not to discuss the individual results presented in these tables, but to attempt a mechanistic overview that relates to their findings. Crucially, there are a number of reasons why oncolytic virus therapy in combination with chemotherapeutic agents, or other anticancer treatments, will be beneficial. Firstly, the mode of action of oncolytic viruses is completely different from chemotherapeutics and they are not, therefore, in direct competition. Secondly, oncolytic cell killing is independent of the many genomic alterations that lead to drug-resistant tumors and so may be effective even in drug-resistant cells.

The most widely used method of studying drug/drug (or virus/drug) interactions between two modalities in vitro is using the methods of Chou and Talalay.[26,28] This type of analysis is one of the few available that identifies beneficial interactions based on an extrapolated equation. The possibility of predicting a false positive is minimized as the analysis takes into account both the potency (the IC_{50} [half the maximal inhibitory concentration] or the LD_{50} [median lethal dose]) and the slope of the dose effect curves (*m*-value) in the precise analysis of two therapeutic combinations. The method defines the expected additive effect of two (or more) agents and quantifies synergy or antagonism by way of how different the measured effect is from the expected additive effect. The equations are detailed elsewhere.[26,28,29] Interpretation of the combination index (CI) values are defined as: CI =1 indicates an additive effect; a CI of <1 indicates synergy; and a CI >1 indicates antagonism. Synergy is the working together of two agents to produce a result greater than the sum of their individual effects, while antagonism is less than that of an additive effect.

Table 2 Advantages of oncolytic virotherapy

Feature	Advantage
Replicates within tumor cells to increase viral dose	Amplification leads to oncolysis in cells beyond those initially infected Increases therapeutic index
Replicates only within tumor cells	Minimal toxicity to normal tissues
Can be used safely with other cancer treatments and may have synergistic effect	Increased efficacy of combined treatment
Can also be engineered or armed to carry a wide variety of transgenes to enhance the therapeutic effect such as prodrugs or inducers of immunological response	Dual effect of viral oncolysis and the added effect of the prodrug or immune stimulator
Some evidence that oHSV are capable of targeting and eliminating cancer stem cells	Eliminates the population of cells that are often resistant to chemotherapy and radiotherapy

Abbreviation: oHSV, oncolytic herpes simplex virus.

Table 3 Main cellular and viral pathways activated upon viral infection

Name of HSV-1 protein	Pathway
Vhs	Inhibits IRF3 and NF-kB Inhibits IFN-induced STAT1 nuclear accumulation and phosphorylation Inhibits eIF2α phosphorylation
ICP34.5	Downregulates MHC class II cell surface expression Inhibits eIF2α phosphorylation
ICP0	Inhibits IRF3/IRF7 to repress ISG production Disrupts ND10 domains Degrades TLR adaptor proteins MyD88 and Mal
ICP27	Inhibits IRF3 and NF-kB Inhibits IFN-induced STAT1 nuclear accumulation Inhibits eIF2α phosphorylation
US11	Prevents eIF2α activation via an interaction with PKR
US3	Controls TLR3 RNA levels

Abbreviations: HSV-1, herpes simplex virus 1; ICP, infected cell polypepetide; IRF3, interferon regulatory factor 3; IRF7, interferon regulatory factor 7; NF-κB, nuclear factort kappa light chain enhancer of activated B cells; IFN, interferon; STAT1, signal transducer and activators of transcription 1; eIF2α, eukaryotic initiation factor 2; Vhs, virion host shutoff protein; MHC, major histocompatibility complex; ND10, nuclear domain 10; TLR, toll like receptor; MyD88, myeloid differentiation primary gene (88); Mal, myelin and lymphocyte protein; ISG, interferon stimulated gene; RNA, ribonucleic acid.

Table 4 Oncolytic viruses and chemotherapeutic agent

oHSV	Drug	Cell line	Cancer type	In vitro	In vivo	Reference
HSV1716	Cisplatin	UM_SCC	HNSCC	Additive	ND	114
		14CUM-SCC 22A	HNSCC	Additive	ND	
		UM-SCC 22B	HNSCC	Additive	ND	
HSV1716	Cisplatin, doxorubicin, mitomycin C, methotrexate	NCI-H460	NSCLC	Additive	ND	27
NV1066	Cisplatin	H-2452, H-Meso,	MPM	Synergistic	ND	41
		H-2373, H-28		Synergistic	ND	
		JMN, Meso-9		Synergistic	ND	
		MSTO-211H		Synergistic	ND	
		VAMT,		Additive	ND	
		H-2052		Additive	ND	
		Meso-10		Additive	ND	
G207	Cisplatin	SCC-25/CP	HNSCC	No effect	ND	123
		Sq20B		ND	No effect	
		UMscc-38		ND	Additive to synergistic	
G47Δ	Cisplatin	LNCaP	Prostate cancer	Antagonistic	ND	89
OncoVex-GALV/CD	Cisplatin	EJ	Bladder transitional carcinoma	Antagonistic	ND	65
		T24		Antagonistic	ND	
		TCCSUP-G		Antagonistic	ND	
rRp450 (CYP2B1)	Cyclophosphamide	Rh30	Alveolar rhabdomyosarcoma	ND	Enhanced	54
G47Δ	Doxorubicin	LNCaP	Prostate cancer	Antagonistic	ND	89
G207	Doxorubicin	KAT4	Anaplastic thyroid cancer	Additive	Enhanced	87
		DRO90-1		Additive	ND	
G47Δ	Docetaxol	LNCaP	Prostate cancer	Synergistic	Enhanced	89
		DU145		Synergistic	ND	
G207	Erlotinib	STS26T	MPNST	Additive	Not enhanced	94
G47Δ	Etoposide	LNCaP	Prostate cancer	Antagonistic	ND	89
G207	Fluorodeoxyuridine	HCT8	Colon cancer	Synergistic	ND	42
G207	5-fluorouracil	KIGB-5 (murine)	Gallbladder	Enhanced	Enhanced (Syrian hamster)	44
		MKN45 (human)	Gastric cancer	Enhanced (viral replication)	Enhanced (SCID mouse)	
NV1020	5-fluorouracil	HT29	Colon cancer	Enhanced	ND	45
		WiDr	Colon	Enhanced	ND	
		HCT116	Colon	Enhanced	ND	
		CT-26	Colon	ND	Enhanced	
NV1066	5-fluorouracil	Hs 700T	Pancreatic cancer	Synergistic	ND	39
		PANC-1 and PaCa-2	Pancreatic cancer	Synergistic	ND	
OncoVex-GALV/CD	5-fluorouracil	A549, H460	Lung cancer	Enhanced	ND	124
		CAPAN-1, MIA PACA-2, BXPC-3	Pancreatic cancer	Enhanced	ND	
		HCT-116, HT-29, SW620	Colon cancer	Enhanced	ND	
		9L LacZ (rat)	Gliosarcoma	ND	Enhanced	
NV1066	Gemcitabine	Hs 700T	Pancreatic cancer	Synergistic	ND	39
		PANC-1 and PaCa-2	Pancreatic cancer	Synergistic	ND	
R3616	Gemcitabine	CAPAN1 and PaCa-2	Pancreatic cancer	ND	Enhanced both cell lines	64
hrR3		SW1990	Pancreatic cancer	ND	Not enhanced	
OncoVex-GALV/CD	Gemcitabine	EJ	Bladder transitional carcinoma	Antagonistic	ND	65
		T24		Synergistic	ND	
		TCCSUP-G		Antagonistic	ND	
		KU19-9		Antagonistic	ND	
HF10	Gemcitabine	CT26	Murine colorectal model	Antagonistic if given together Synergistic ifinjected GEM is pretreatment	Enhanced effect in both tumor and distal tumor	88

(Continued)

Table 4 (*Continued*)

oHSV	Drug	Cell line	Cancer type	In vitro	In vivo	Reference
NV1020	Irinotecan (SN38)	HT29 and WiDr	Colon cancer	Enhanced	ND	45
		HCT-116		Enhanced	ND	
MGH2	Irinotecan (SN38)	Gli36ΔEGFR	Glioma	Enhanced	Enhanced	59
		U87ΔEGFR		Enhanced	ND	
		U251		Enhanced	ND	
		T98G		Enhanced	ND	
G207	Mitomycin C	OCUM-2MD3	Gastric cancer	Synergistic	Enhanced	36
		MKN-45-P		Synergistic	ND	
NV1066	Mitomycin C	KU19-19	Bladder transitional carcinoma	Synergistic	ND	126
		SKUB		Synergistic	ND	
OncoVex-GALV/CD	Mitomycin C	EJ	Bladder transitional carcinoma	Synergistic	ND	65
		T24		Synergistic	ND	
		TCCSUP-G		ND	ND	
		KU19-9		Synergistic	ND	
NV1020	Oxaliplatin	HT29 and WiDr	Colon cancer	Enhanced	ND	45
		HCT-116	Colon cancer	Enhanced	ND	
G207	Paclitaxel	KAT4	Anaplastic thyroid cancer	Synergistic	Enhanced	87
		DRO90-1		Synergistic	ND	
NV1023	Paclitaxel	KAT4	Anaplastic thyroid cancer	Synergistic	ND	87
		DRO90-1		Additive	ND	
G47Δ	Paclitaxel	LNCaP	Prostate cancer	Synergistic	ND	89
		DU145		Synergistic	ND	
MGH2	Paclitaxel	MDA-MB-435S	Mammary carcinoma	ND	Enhanced	127
G207	Temozolomide	U87	Malignant glioma	Synergistic	Enhanced	128
		U87-dnp53		Synergistic	ND	
		U373		Synergistic	ND	
		T98		Synergistic (with O6-benzylguanine)	ND	
		U87MGMT		Synergistic (with O6-benzylguanine)	ND	
G47Δ	Temozolomide	GBM13	Glioma stem cells (TMZ resistant/ MGMT+ve)	No synergy	ND	37
		BT74		No synergy	Not enhanced (enhanced in the presence of + O6-benzylguanine)	
		U87MG	Glioma	No synergy	ND	
		T98	Glioma	No synergy	ND	
		GBM4	Glioma stem cells (TMZ sensitive/ MGMT-ve)	Synergistic	ND	
		GBM6		Synergistic	ND	
		GBM8		Synergistic	Enhanced	
G207	Vincristine	KFR	Rhabdomyosarcoma	Enhanced	Enhanced	90
		KF-RMS-1		Enhanced	Enhanced	
NV1042	Vinblastine	CWR22	Prostate	Synergistic	Enhanced	78
		PC3		Synergistic	ND	

Abbreviations: MPM, malignant pleural mesothelioma; oHSV, oncolytic herpes simplex virus; TMZ, temozolomide; HNSCC, head and neck squamous cell carcinoma; ND, not done; MPNST, malignant peripheral nerve sheath tumor; GEM, gemcitabine; MGMT, methylguanine DNA ethyltransferase.

Table 5 Oncolytic viruses and mTOR inhibitors

oHSV	Drug	Cell line	Cancer type	In vitro	In vivo	Reference
Baco-1	Rapamycin	HepG2	HCC	No effect	ND	46
		HuH-7	HCC	No effect	ND	
		MDA-MB-231	Breast cancer	No effect	ND	
		EC9706	Esophageal	Additive	Additive	
		MCF-7	Breast cancer	Additive	ND	
		HeLa	Cervical	Additive	ND	
MG18L	BEZ235	GBM4	Glioma stem cells	No effect	ND	85
		GBM8		No effect	ND	
		GBM13		Synergistic	ND	
		BT74		No effect	ND	

Abbreviations: oHSV, oncolytic herpes simplex virus; HCC, hepatocellular carcinoma; ND, not done.

Table 6 Oncolytic viruses and PI3K inhibitors

oHSV	Drug	Cell line	Cancer type	In vitro	In vivo	Reference
R7041	LY294002	U87	Glioma	Synergistic	Enhanced	86
MG18L	LY294002	GBM4	Glioma stem cells	Synergistic	ND	85
		GBM8		No effect	ND	
		GBM13		Synergistic	ND	
		BT74		Synergistic	Enhanced	
		U87	Glioma	Synergistic	ND	
		T98G		Synergistic	ND	
MG18L	GDC-0941	GBM4	Glioma stem cells	Synergistic	ND	85
		GBM8		No effect	ND	
		GBM13		No effect	ND	
		BT74		Synergistic	ND	
		U87	Glioma	Synergistic	ND	
		T98G	Glioma	Synergistic	ND	

Abbreviations: oHSV, oncolytic herpes simplex virus; ND, not done; PI3K, phosphatidylinositide 3-kinases.

Chou and Talalay[26] analysis can also be used effectively in vivo, but it is more common practice, as reported in the literature, to look for differences in tumor growth between treatment groups and to use analysis of variance or *t*-tests to determine if the differences (often either tumor volume or length of survival) between groups are significant. Information on synergy and/or enhanced efficacy of combinations will also come from clinical studies. Most patients that take part in new cancer therapy trials have already had, or are currently being treated with, the standard treatment for their particular disease, and it will be interesting to see if any group treated with oHSV and another agent respond better or worse than predicted. There are a number of different ways in which an oHSV in combination with an anticancer drug can be synergistic and these are discussed below.

Compounds that increase the replicative capacity of the virus

Oncolytic HSV have selective replication competence in cancer cells and, by increasing the replicative capacity of the virus within those cells, the number of progeny viruses produced during a cycle of infection could be increased (Figure 2).

Differentiating inducing agent hexamethylene bisacetamide (HMBA) has been shown to improve viral yield, with up to a 10,000-fold increase in vitro for an ICP34.5 null virus, R849, at low MOI (multiplicity of infection). HSV immediate early gene expression (Figure 4 shows the basic HSV replication cycle) was also increased with HMBA.[30] Mice treated with both HMBA and R849 virus had significantly smaller tumor burden and survived longer than either virus or HMBA treatment alone, with increased levels of HSV transcripts of immediate early, early, and late genes in the combination treatment group. This suggests HMBA may increase and/or activate cellular proteins such as transcription factors, which act to improve viral yield. HMBA is a drug that was thought to have some potential as a stand-alone anticancer agent; however, the level of drug required for such anticancer activity could not be achieved in patients.[31] In the study with oHSV, a much lower dose of drug was able to be used; one which could easily be achieved in patients and potentially would act as a promoting agent for oncolytic therapy.

Table 7 Oncolytic viruses and HDAC inhibitors

oHSV	Drug	Cell line	Cancer type	In vitro	In vivo	Reference
G47Δ	Trichostatin A	U87	Glioma	Synergistic	Enhanced	65
		T98		Synergistic	ND	
		SW480	Colon cancer	Synergistic	Enhanced	
		HeLa	Cervical cancer	Synergistic	ND	
		MCF-7	Breast cancer	Additive	ND	
R849	Trichostatin A	SAS	Oral SCC	Enhanced	ND	132
		Ca9-22		ND	ND	
		HSC		ND	ND	
rQNestin34.5	Valproic acid	U251	Glioma	ND	ND	133
		U87Δ EGFR		ND	Enhanced	

Abbreviations: oHSV, oncolytic herpes simplex virus; SCC, squamous cell carcinoma; ND, not done; HDAC, histone deacetylase.

Table 8 Oncolytic viruses and others

oHSV	Drug	Cell line	Cancer type	In vitro	In vivo	Reference
OncdSyn	Thalidomide	4T1	Breast	ND	Enhanced	134
R849	Hexamethylene bisacetamide	Ca9-22	Oral SCC	Enhanced	ND	30
		SAS		Enhanced	ND	
		F1		Enhanced	Enhanced	

Abbreviations: oHSV, oncolytic herpes simplex virus; SCC, squamous cell carcinoma; ND, not done.

Another mechanism for increasing viral yields may be to temporarily block apoptosis. Upon viral infection, one of the cellular host responses is to induce apoptosis in infected cells and in cells surrounding infected cells (Figure 3) in order to limit the ability of the virus to replicate and spread. Therefore, by blocking apoptosis temporarily, there is the potential for improving the propagation of viral progeny, maximizing the lateral spread of virus and increasing tumor destruction. Wood and Shillitoe[32] reported on increased viral replication in the presence of zVAD-fmk; a pan caspase inhibitor that has previously been shown to prevent HSV-1-induced apoptosis.[33] The authors showed that the inhibitor increased levels of replication in an ICP34.5 null mutant back to the levels of wild type HSV-1. Stanziale et al[34] also reported increased apoptosis in cells that neighbored NV1066-infected cells and could mitigate this effect with treatment with an inhibitor of apoptosis: N-acetylcysteine. This suggests that the increased viral yield seen with the caspase inhibitors is likely to be due to neighboring noninfected but alarmed cells being prevented from initiating apoptosis and, therefore, become lytically infected with virus.[32,34] Eisenberg et al[35] reported that hyperthermia potentiates oncolytic viral killing. After hyperthermic insult, the heat shock protein Hsp72 is upregulated, which inhibits cellular apoptosis, thereby allowing increased viral replication and, in turn, enhanced tumor kill. This finding has great potential as, in a clinical setting, the application of heat is likely to be noninvasive and relatively toxicity free.

Compounds that increase cell permissiveness to oHSV

Many chemotherapeutic drugs are DNA damaging agents and, following exposure to such agents, cells upregulate their DNA damage repair pathways. Such upregulation appears to be beneficial for oncolytic viral replication; mitomycin C,[36]

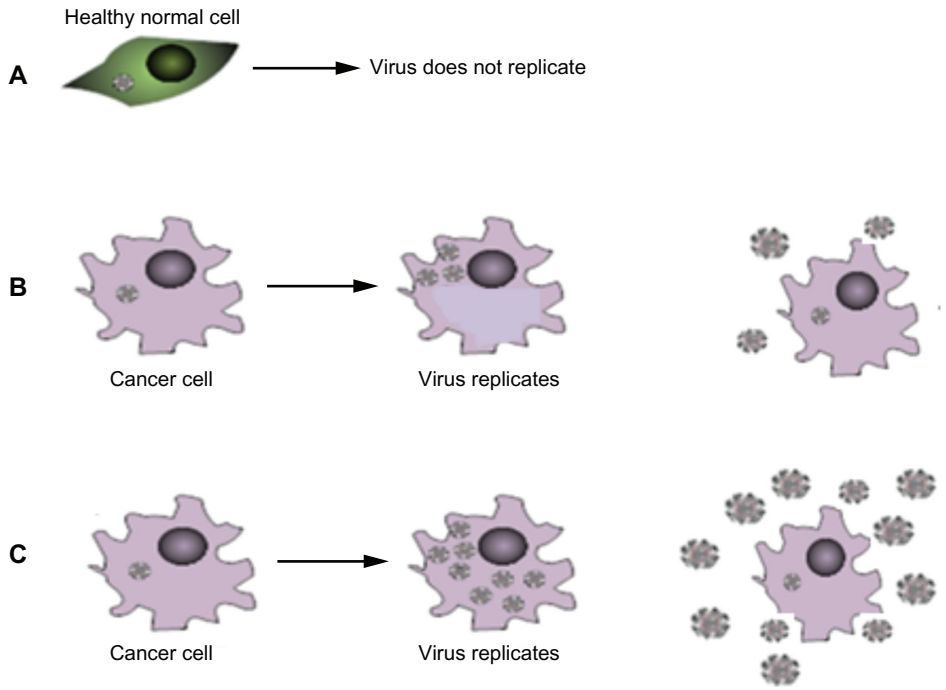

Figure 2 Increasing replicative capacity of the virus: (**A**) in normal cells the virus does not replicate. (**B**) In a cancer cell the virus replicates, lyses the cell and produces viral progeny that go on to infect further cancer cells. (**C**) In the presence of certain drugs the virus can produce more viral progeny. Upon lysis more progeny virus are released – potentially increasing the number of cells that can be infected.

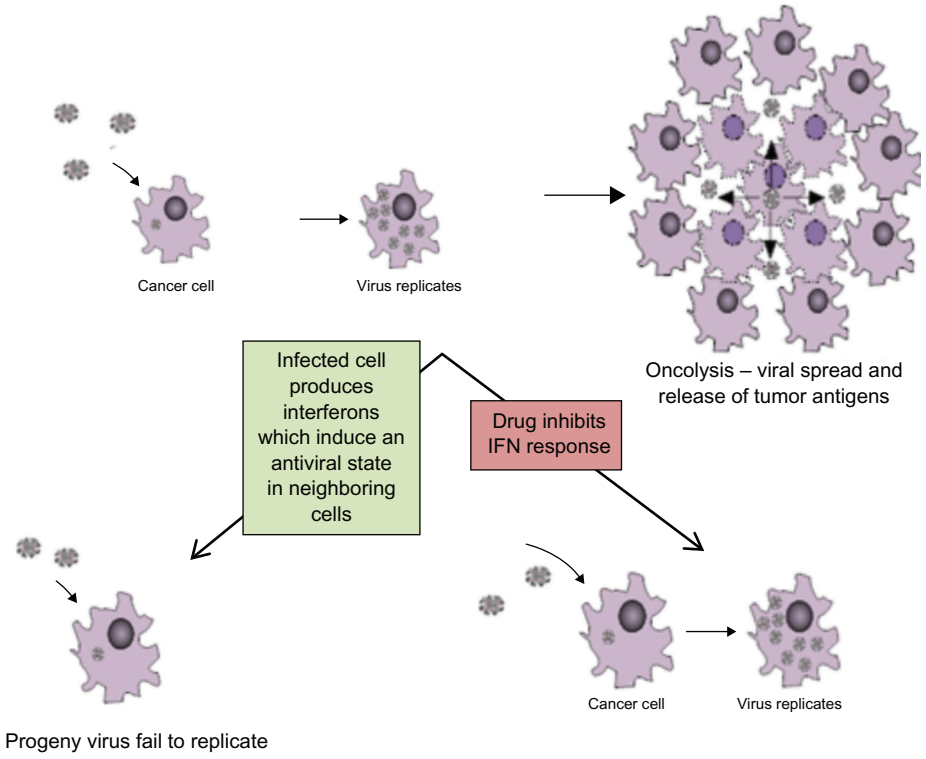

Figure 3 Anti-viral host response mediated by IFN (interferon) induces apoptosis of surrounding cells. By using drug to block innate antiviral defence mechanism the infected cell will not signal other nearby cells to 'warn' them about the virus, hence viral replication will occur.

temozolomide,[37,38] and 5FU[39] have all been shown to increase oncolytic HSV replication.

Growth arrest and DNA damage-inducible protein GADD34 is induced by stressful growth arrest conditions and treatment with DNA-damaging agents. The carboxyl terminal of GADD34 bears significant homology with the virulence factor ICP34.5, which is deleted in some oHSV, eg, HSV1716, NV1066, R3613, and T-Vec (Table 1). Previous studies[40] have

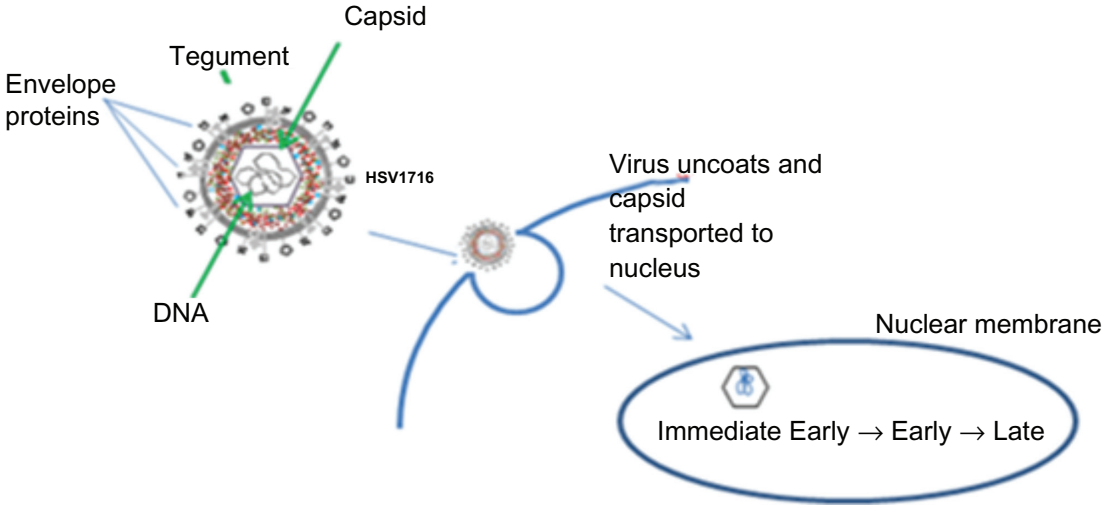

Figure 4 Herpes simplex virus (HSV) replication cycle HSV-1 is a double stranded DNA virus which encodes for around 100 transcripts and contains three main structural components. The central capsid (or nucleocapsid) contains the viral DNA. This is surrounded by an envelope. The tegument is located between the envelope and the capsid. HSV enters the host cell at either the cell surface or via pH dependent endocytosis through a process involving envelope glycoproteins. The tegument proteins are released into the cell and the capsid is transported to the nucleus where viral DNA is released into the nucleus. There are three classes of viral genes that are transcribed and translated in a specific order: Immediate Early (IE) genes, which encode for proteins that promote expression of viral genes and also have a role in innate immune invasion, Early (E) are responsible for the replication of viral DNA and lastly Late (L) genes which include capsid, tegument and envelope proteins.

shown that the carboxyl terminus of GADD34 can substitute for ICP34.5 in preventing premature shutoff of protein synthesis, and ICP34.5 null mutants can use the host cell GADD34 protein for viral replication. Thus, the presence of GADD34 in tumor cells following treatment with a DNA damaging agent would increase the number of cells permissive to oHSV infection and increase the viral spread through the tumor. Indeed, when GADD34 small interfering RNAs (siRNAs) were added to block GADD34 expression after treatment with a DNA damaging agent (cisplatin), the previously observed synergy with the oHSV NV1066 and cisplatin was abolished.[41]

Another potential mechanism for synergy with some oHSV is upregulation of cellular RR by DNA-damaging chemotherapeutic agents.[42] High throughput screening has been reported to identify small-molecule compounds that augment the replication of HSV G47Δ,[43] and, of the 2,460 compounds screened, six compounds were identified and subsequently validated for enhanced G47Δ replication. Two of these compounds, dipyridamole and dilazep, interfered with nucleotide metabolism by potently and directly inhibiting the equilibrative nucleoside transporter-1 and were dependent on HSV mutations in ICP6, the large subunit of RR. Equilibrative nucleoside transporter-1 antagonists are thought to augment oHSV replication in tumor cells by increasing cellular RR activity.[43] As oHSV with *UL39* deletions can only replicate in cells with active cellular RR, increasing cellular RR will improve viral replication.

Nakano et al[44] reported an upregulation in RR in tumors mediated by 5FU that augmented the therapeutic effect of G207. 5FU was also found to be synergistic both in vitro and in vivo with oHSV NV1020 (an oHSV with intact ICP6),[45] suggesting the effects of 5FU are not limited to upregulation of RR. The authors speculated that the synergy was in part due to the cells being sensitized to 5FU as the virus caused the cells to arrest in S phase of the cell cycle. They further speculated that the reduction in viral progeny could be due to the immune IFN (interferon)-γ response as well as the 5FU-induced upregulation of cell death via molecules such as TRAIL (TNF [tumor necrosis factor] related apoptosis-inducing ligand) and Fas ligand.

Rapamycin markedly increased the yield and dissemination of oHSV in semipermissive tumor cells both in vitro and in vivo but had no additional effect in cell lines that are permissive to the ICP34.5 null mutant oHSV Baco1.[46] The reason behind the observation is still unclear; however, inhibitors of the mTOR (mammalian target of rapamycin) signaling pathway increase permissiveness of resistant tumor cells to oncolytic myxoma virus,[47] vesicular stomatitis virus,[48] adenovirus,[49] and cytomegalovirus,[50] suggesting that the mTOR signaling pathway has an important role to play in virotherapy.

Compounds that modulate the immune system

The immune response to oncolytic viral therapy is an essential factor determining the success of oHSV as an antitumor agent; it can be a hindrance if it causes premature viral clearance, or could be seen as a positive, with the virally infected tumor becoming a target for clearance by the immune system.

The immune response to viral infection is beyond the scope of this review, but for an excellent insight into this field see Paludan et al.[22] Briefly, the immune reaction to a viral infection (oncolytic or otherwise) is a multipronged response. Very quickly upon infection, the innate immune response recruits natural killer (NK) cells, macrophages, and neutrophils to the site of infection and mediates a nonspecific viral clearance. NK cells appear to be an important player in the response to viral infection; patients with naturally occurring NK cell deficiencies (despite there being numerous different mutations that cause such deficiencies) have severe and recurrent herpes virus infections.[51] NK cells, activated by macrophages secreting IL-12, mediate the lysis of virally infected cells by releasing cytotoxic granules containing lytic enzymes and by binding to apoptosis-inducing receptors on the infected cell. In addition, NK cells secrete IFN-γ, which activates further macrophages and, consequently, orchestrates the downstream adaptive immune response.

The oncolytic HSV rQNestin34.5 (ICP34.5 expression controlled by the nestin promoter) has been shown to induce a rapid recruitment of NK cells to orthotopic human glioblastoma xenografts with subsequent killing of the oHSV-infected xenograft cells by activated macrophages. Depletion of NK cells improved the oHSV efficacy in these glioblastoma models, further indicating the importance of the NK cells.[52] Previous studies have demonstrated that inhibition of the innate immune response using cyclophosphamide[53–56] or macrophage depletion[57] enhances oHSV replication and efficacy. An oHSV variant, rRp450, with deleted ICP6 and incorporated cytochromeP450 transgene for direct cyclophosphamide activation has been described, and the virus enhances the antitumor effects of cyclophosphamide.[18,54,58,59]

Another key event in the immune response to viral infection is the secretion of IFN-γ (for an extensive review see Roizman[40] and Bazan-Peregrino et al[60]). The cytokine IFN-γ, or type II interferon, is critical for innate and adaptive immune response to viral infection, partly from its ability to inhibit

viral replication directly, but, more importantly, also from its immunostimulatory and immunomodulatory effects. IFN-γ is produced predominantly by NK cells as part of the innate immune response, and by cluster of differentiation (CD)4+ T helper (Th)1 and CD8 cytotoxic T lymphocyte (CTL) effector cells once antigen-specific immunity develops.

Histone deacetylase inhibitors (HDIs) are a class of compounds that appear to benefit HSV oncolysis, possibly via suppression of innate immune responses. Histone deacetylases (HDACs) have pleiotropic effects on cells through deacetylation of proteins, including histones, which then alter the epigenome and transcription profiles. Numerous HDACs have been targeted for drug discovery for cancer therapies, either for use as a single agent or in combination with chemotherapeutic agents. Pretreatment with the HDI valproic acid was shown to enhance the oncolytic virus MGH2 and rQNestin34.5 replication and spread in tumors, and extended the survival of mice bearing intracerebral tumors.[52,61] The authors attributed the synergy between HDIs and oHSV to inhibition of type I interferon responses that would usually restrict viral gene expression and replication.

Drugs that cause downregulation of the innate immune response can be synergistic with oncolytic viruses but there is also evidence of the immune response enhancing tumor clearance.[62] Benencia et al[63] reported that oHSV therapy was less effective in murine metastatic melanoma models lacking NK and T cell subsets. Similarly, HSV1716-induced expression of IFN-γ inducible chemokines was accompanied by a significant increase in the number of NK and $CD8^+$ cells in the tumor microenvironment in a syngeneic ovarian carcinoma model.[59,63]

Synergy has also been reported with oHSV and compounds that increase IFN-γ production.[64] The authors found that pretreating tumor cells with gemcitabine before oHSV significantly reduced tumor growth in vivo. Pretreatment was necessary as the drug itself induces early termination of DNA synthesis, which prevents replication of oncolytic viruses.[39,64–66] Gemcitabine selectively kills myeloid-derived suppressor cells, which inhibit IFN-γ production by CD8+ cells. So, when myeloid-derived suppressor cells themselves are killed, CD8+ T cells will secrete higher levels of IFN-γ, thus directing more T cells to tumor sites, which results in an improved antitumor response. In addition, IFN-γ can change the tumor microenvironment in terms of macrophages phenotype. Macrophages are classified as m1 (classically activated) or m2 (alternatively activated). During tumor progression there is a switch from m1- to m2-like phenotype that is believed to allow the tumor cells to avoid the immune system. Higher levels of IFN-γ can change the macrophage phenotype back to m1, resulting in the cancer cells being more likely to be tagged for destruction by the immune system.[64]

Recently, a number of immunotherapeutic agents have been approved as cancer treatments. Ipilimumab, a monoclonal antibody that blocks the CTL-associated antigen 4 receptor, which would normally inhibit cytotoxic T lymphocyte, for example, is approved for use in advance metastatic melanoma.[67,68] It is by blocking the CTL-associated antigen 4 receptor that CTLs are activated and can recognize and destroy cancer cells. As the presence of an oncolytic virus within a tumor will make the tumor more antigenic, there is good reason to think that the combination of oncolytic virus and immunotherapy will be synergistic and, indeed, there are many reports of improved efficacy of oHSV engineered to express genes that make immunomodulatory proteins including IL-12, IL-24, IL-4, RANTES (Regulated on Activation, Normal T cell Expressed and Secreted), CD80, and IFNα.[68] Granulocyte-macrophage colony-stimulating factor, which generates an antitumor response by the recruitment and differentiation of activating dendritic cells in the tumor microenvironment, has been inserted successfully into T-Vec,[69,70] and a clinical study investigating T-Vec in combination with ipilimumab is underway,[71] with primary results expected in summer 2016.

Immunomodulatory drugs highlight the complexities of potential interactions between oHSV and anticancer agents, with synergy reported with drugs that inhibit or upregulate the immune system. It is likely that drugs that inhibit the very early innate immune response will allow the virus longer to enter cells and undergo initial viral replication, increasing the spread of the virus. Drugs that act by boosting later immune responses, such as upregulating T cells, mean that the infected tumor cells and potentially uninfected neighboring tumor cells are more likely to be targeted for destruction by the immune system. It will be interesting to see if downregulating innate immunity by HDIs, for example, and upregulating T cells by gemcitabine, would result in further synergistic effects when combined with an oncolytic virus. To date, no triple combinations have been reported in the literature, probably due to the increasing complexity of such experiments.

Compounds that alter the tumor microenvironment

Angiogenesis is the formation of new blood vessels and, as tumors need blood vessels to grow and spread, inhibitors of angiogenesis, which prevent the formation of new blood vessels, could potentially prevent or slow the growth or spread

of tumors. Unlike chemotherapeutic agents, angiogenesis inhibitors will not kill cancer cells directly but instead prevent tumors from growing, so potentially, in order to completely eradicate a tumor, an antiangiogenic drug would have to be given in combination with a modality that kills cancer cells, such as an oncolytic virus.

Vascular endothelial growth factor (VEGF) is a key component in tumor angiogenesis and is overexpressed in many human tumors. It has numerous effects on tumor vasculature such as increased vasodilation and permeabilization, and inhibitors of VEGF, such as Avastin®, sorafenib, and sunitinib, appear to "normalize" tumor vasculature, potentially enhancing localization of systemic oncolytic virus. ICP34.5 null oHSV infectivity and cytotoxicity were diminished under hypoxic conditions (when the cells are deprived of oxygen) in several glioblastoma xenolines, which are cell lines maintained by xenograft passage.[69] Normalization of the blood vessels by antiangiogenic agents may reduce hypoxia within the tumor microenvironment and potentially improve oHSV replication. However, other studies have shown improved oHSV replication in hypoxic conditions.[70–73] Bevacizumab (Avastin®), a monoclonal antibody against VEGF A, had no effect on the spread or replication of oHSV in vitro. However, in vivo, in several studies using different xenograft models,[74,75] groups of mice receiving the dual therapy of both oHSV and Avastin® had tumors that were significantly smaller than tumors from either treatment alone. Results from these studies indicated that Avastin® improved replication and spread of the oHSV within the xenograft microenvironment. Although cytotoxic in vitro, in some xenograft models rRp450 had only mild antitumor effects.[76] The host inflammatory response to rRp450 therapy was found to induce an acute neutrophil infiltrate, a relative decrease of intratumoral macrophages, and a myeloid cell-dependent upregulation of host-derived VEGF. Bevacizumab and r84 (which selectively inhibit binding to VEGF receptor 2 but not VEGF receptor 1) enhanced the antitumor effects of rRp450 therapy, in part due to decreased angiogenesis. However, although neither bevacizumab nor r84 increased virus production or affected neutrophil infiltration, both partially mitigated virus-induced depletion of macrophages. Therefore, the enhancement in efficacy with the combination of oHSV therapy and anti-VEGF antibodies appears to be in part due to modulation of host inflammatory reaction to virus.

Vinblastine, a microtubule disrupting agent that has been shown to inhibit angiogenesis in humans[77] and, in combination with the oHSV NV1042, showed increased antitumor and antiangiogenic effects in vivo in prostate cancer models,[78] provides further evidence that the combination of an antiangiogenic agent and an oncolytic virus may have clinical benefit. However, to the best of our knowledge, there are no preclinical published studies of oHSV in combination with small molecule VEGF receptor inhibitors such as sorafenib or sunitinib.

HSV DNA replication occurs in discrete compartments in the nucleus that assemble as prereplicative sites with viral DNA and the HSV DNA binding protein ICP8. HSV DNA polymerase and cellular factors are then recruited to these compartments for use in viral replication. The DNA damage and repair pathways repair the damage to the cancer cell DNA caused by treatment with DNA-damaging drugs such as temozolomide (TMZ). However, in the presence of oHSV infection, key components of these pathways are sequestered into discrete compartments for use in viral replication, hence are not available to repair the damage caused by drugs. Thus, the damage, in terms of number of cancer cells killed by a specific amount of drug, is greater in the presence of oHSV.[37]

Cellular kinases play a key role in the regulation of signaling events that govern multiple pathways affecting growth, proliferation, migration, and angiogenesis. These include PI3K (phosphatidylinositide 3-kinases)-Akt-mTOR and mitogen-activated protein kinases pathways, which are often mutated in cancer cells to support unchecked cellular replication. Inhibition of these pathways could potentially reduce tumor growth, and this is reflected in the intensive drug development looking for PI3K-Akt-mTOR and mitogen-activated protein kinases inhibitors. For example, 80% of glioblastomas are having genetic alterations in the PI3K-Akt-mTOR pathways and there are at least 10 different inhibitors in development.[79] However, due to the high level of redundancy and cross regulatory feedback loops, monotherapy may be unlikely to have significant clinical efficacy;[80] for example, rapamycin only reduces mTOR activity for 12 hours before another kinase substitutes and reengages the mTOR network.[81] Furthermore, such inhibitors are likely to be cytostatic: they will stop the cancer cells from growing or dividing but will not eradicate them.

The PI3K-Akt-mTOR pathway is also important in viral replication (for a full review see Terada et al[61] and Buchkovich et al[82]). Upon infection, viruses frequently activate this pathway to benefit from the survival signaling associated with Akt activation. One of the downstream effectors of activated Akt is the mTOR kinase, a component of the mTOR complexes (mTORC) 1 and 2. Activated mTORC1 is crucial for the maintenance of cap-dependent translation which is

required by most mammalian DNA viruses and many RNA viruses. mTORC2 is less well understood, but is thought to have roles in Akt phosphorylation and the organization of the actin cytoskeleton. It would therefore seem reasonable to assume that inhibitors that block the function of mTOR or PI3K would not only block translation of cellular proteins but would drastically reduce the ability of viruses to replicate by virtue of stopping their cap-dependent translation. Theoretically, PI3K and mTOR inhibitors would be antagonistic if used in combination with oncolytic viruses. The literature, however, reveals diverse results that vary depending on the specific virus, the specific inhibitor, and the status of the cells used.

Breitbach et al[83] found that compounds such as rapamycin, which blocks the activation of mTOR, and PD098059, which blocks the activation of MAP (mitogen-activated protein) kinase, did not affect the ability of oHSV R3616 to replicate in pancreatic tumor cells. Treatment with the inhibitor LY294002, which inhibits the PI3K pathway, prevented the replication of R3616. Similarly, synergy was not observed between LY294002 and the ICP34.5 null oHSV, but was observed with oHSV mutants with a Us3 mutation.[84] The gene product of Us3 protects virus-infected cells from apoptosis; a cellular pathway that is often dysfunctional in tumors. Thus, Us3 mutants, whose replication would be inhibited by apoptosis in normal cells, would be selective for tumor cells, and the combination treatment of LY294002 and Us3-null oHSV is synergistic due to enhanced apoptosis in the combination treated cells.[85]

Compounds that affect the cell cycle

Strong synergy between oHSV and trichostatin A (an HDAC inhibitor) was observed in a wide range of cancer and proliferating endothelial cell lines but not in normal prostate or quiescent epithelial cells.[86] Unlike other HDIs, the synergy was seen regardless of the dosing sequence of the oHSV (G47Δ) or trichostatin A. The synergy was attributed to reduced cyclin D1 expression in cells that normally have a high level of cyclin D (ie, cancer cells). The combination also inhibited secretion of the angiogenic factor VEGF, which correlated with the decreased vascularity within the tumor in vivo.

Another combination that appears to affect the cell cycle occurs between the oHSV G207 and paclitaxel. Paclitaxel is an approved cancer therapy that stabilizes microtubules and, as a result, interferes with the normal breakdown of microtubules during cell division. In the presence of paclitaxel, chromosomes are unable to achieve metaphase spindle configuration. This inability to form the correct formation blocks the progression of mitosis which in turn triggers apoptosis or the cell to revert to the G phase of the cell cycle without dividing. Despite the G207/paclitaxel combination being synergistic, oncolysis or viral replication was not increased.[87] The authors concluded that they differentially affected cell cycle progression, either by the cells arresting in G1 (virus-mediated) or mitosis (paclitaxel-mediated), a combination that served to increase apoptosis further. Paclitaxel also showed synergy with other oHSV, HF10, and G47Δ, both in vitro and in vivo.[88,89] The oHSV HF10 has been studied alone and in combination with paclitaxel in colon cancer models.[88] In vivo, the combination of HF10 and paclitaxel prolonged survival of mice bearing carcinomatous dissemination of CT26 tumors compared with the control groups. G47Δ also synergized with paclitaxel and the closely related docetaxel to enhance the in vitro killing of LNCap and DU145 prostate cancer cells.[89] Docetaxel-induced accumulation of the phosphospecific mitotic markers op18/stathmin or histone H3 was significantly reduced by G47Δ, and this correlated with enhanced apoptosis and required active virus replication. Another microtubule inhibitor, vincristine, was also shown to be synergistic with oHSV in rhabdomyosarcoma xenografts.[90]

Cheema et al[91] reported synergy with etoposide, an inhibitor of topoisomerase II, and oHSV G47Δ in glioma stem cell xenografts. Gutermann et al[45] found synergy with SN38 (the active metabolite of irinotecan, a topoisomerase I inhibitor) and NV1020 in a panel of human colon carcinoma cell lines in vitro. Synergy with irinotecan and MGH2 (an oHSV with UL39 and -γ34.5 deletions) was also reported in glioma, both in vitro and in vivo.[59]

Other compounds where synergy and/or enhancement is seen but the mechanism is unclear

Although not using an oHSV, Heo et al[92] reported on the first clinical signs of positive interactions between oncolytic virotherapy and standard of care drugs with JX-594 (an oncolytic pox virus) and sorafenib, a small molecule inhibitor of the signaling oncoprotein B-raf and VEGF receptor, which is licensed as a treatment for hepatocellular carcinoma. The authors reported that a number of patients treated with JX-594 and then sorafenib up to 8 weeks later had objective tumor responses (ie, tumor shrinkage) compared to zero in 15 untreated patients matched for age, stage, and sex. Furthermore, they also reported a complete cure in one patient treated with sunitinib, another inhibitor

similar to sorafenib, 8 weeks after JX-594 treatment. As the virus is likely to be cleared from the patient by 8 weeks, the mechanism by which the oncolytic virus can sensitize tumors to these inhibitors is unclear. Interestingly, the patients who have the best responses to sorafenib are those patients who have hepatitis C related hepatocellular carcinoma,[93] suggesting that there may be a therapeutic class effect where viruses sensitize tumors to VEGF receptor inhibitors.

Erlotinib, an epidermal growth factor receptor inhibitor, combined additively with two oHSV, G207, and hrR3 in order to enhance cytotoxicity in vitro in human malignant peripheral nerve sheath tumor cells often associated with Ras/epidermal growth factor receptor hyperactivation; however, this effect did not translate into an in vivo malignant peripheral nerve sheath tumor xenograft model.[94] Thalidomide, which is now approved for use in multiple myeloma patients, was found to have significant benefit in reducing tumor burden in combination with OncdSyn (an NV1020-like oHSV) than either OncdSyn or thalidomide alone in a murine breast cancer model,[95] though the mechanism is unclear.

Conclusion

Oncolytic viruses are a new and emerging treatment for cancer. As they become an established therapy, much attention will have to be paid to the interaction between current standard of care drugs and oncolytic viruses. So far, the signs are encouraging; not only can oHSV be given alongside other cancer treatments, but can actually result in an enhancement of efficacy in reducing tumor burden and improving survival. The majority of virus–drug combinations listed in Tables 4–8 show synergistic, enhanced, or additive effects, but this may in part reflect the fact that antagonistic combinations might not be submitted for publication. Recently, Kulu et al[96] reported on the inhibition of HSV oncolysis in colon and pancreatic cancer cell lines in vitro when combined with 5-FU, irinotecan, or methotrexate. Their studies showed that replication of both ICP6 and/or ICP34.5 deleted oHSV was significantly reduced in HT29 and SW620 (colon) and Capan-2 (pancreatic) cell lines. Others have reported additive/synergistic interactions (with respect to cell killing) between 5-FU, irinotecan, and methotrexate (Table 2) with oHSV in diverse cell lines, including both colon and pancreatic lines. It is conceivable that the drugs can inhibit virus replication but the combined effects of virus and drug act in concert to enhance cell death, and seemingly conflicting results serve to illustrate our poor understanding of such interactions.

Furthermore, the sequence in which the drug and oHSV are given may impact on cell killing. For example, gemcitabine and HDIs such as valproic acid are synergistic when given as a pretreatment to the virus, thus sensitizing the tumor to virus, whereas sorafenib appeared to work better given after oncolytic virus; thus the virus is acting as the sensitizer. Similarly, when oHSV rRp450 was given before Avastin® (bevacizumab) there was a significantly prolonged survival compared to the same combination in reverse order.[74]

Many of the published combination studies examined the effects of combinations in vitro. These identify combinations that enhance cancer cell cytotoxicity. However, many of the interactions between oHSV and drugs either affect the tumor or host biology, and these interactions will only be seen in vivo. The immune system is a key player in the efficacy of any combination treatment; it appears that initial suppressing of the innate immune response in order to allow the virus to undergo replication, then an upregulation of the immune system to clear the virus and tumor, would be a rational strategy in terms of reducing tumor burdens.

The use of patient-derived tumor xenografts, where primary human tumors are transplanted into immune deficient mice within hours after the sample is collected, are increasingly being used to predict the effectiveness of chemotherapeutic drugs in patients. To our knowledge, such models have not been reported for testing combinations of oncolytic HSV together with chemotherapy or targeted drugs, but are likely to be valuable and should provide data that will improve decision making and accelerate development programs for virus/drug combinations.

As preclinical studies progress into the clinical setting, major progress in the understanding of oHSV in combination with other treatments is likely to occur. Early clinical trials usually involve patients who have already exhausted all the available standard treatment options, and even later Phase III trials will often compare standard of care versus standard of care plus oHSV. Such studies should help confirm preclinical findings on useful virus/drug combinations and hopefully bring benefit to cancer sufferers.

Disclosure

Lynne Braidwood, Joe Conner, and Alex Graham are employees of Virttu Biologics Ltd. The authors report no other conflicts of interest in this work.

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