

Targeting tumor vasculature through oncolytic virotherapy: recent advances

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Abstract: The oncolytic virotherapy field has made significant advances in the last decade, with a rapidly increasing number of early- and late-stage clinical trials, some of them showing safety and promising therapeutic efficacy. Targeting tumor vasculature by oncolytic viruses (OVs) is an attractive strategy that offers several advantages over nontargeted viruses, including improved tumor viral entry, direct antivascular effects, and enhanced antitumor efficacy. Current understanding of the biological mechanisms of tumor neovascularization, novel vascular targets, and mechanisms of resistance has allowed the development of oncolytic viral vectors designed to target tumor neovessels. While some OVs (such as vaccinia and vesicular stomatitis virus) can intrinsically target tumor vasculature and induce vascular disruption, the majority of reported vascular-targeted viruses are the result of genetic manipulation of their viral genomes. Such strategies include transcriptional or transductional endothelial targeting, “armed” viruses able to downregulate angiogenic factors, or to express antiangiogenic molecules. The above strategies have shown preclinical safety and improved antitumor efficacy, either alone, or in combination with standard or targeted agents. This review focuses on the recent efforts toward the development of vascular-targeted OVs for cancer treatment and provides a translational/clinical perspective into the future development of new generation biological agents for human cancers.

Keywords: vascular targeting, oncolytic virus, tumor angiogenesis

Introduction

The oncolytic virotherapy field has significantly expanded in the last decade, with ~190 clinical trials using new viral vectors for the treatment of human malignancies, of which ~11 are in advanced stages of development. As oncolytic viruses (OVs) have an intrinsic ability to infect, replicate in, and induce cytotoxicity in a cancer selective manner,^{1,2} they offer a potential advantage over standard anticancer therapies. OVs allow the introduction of therapeutic genes³ or modifications in the viral genome to modulate viral tropism and improve virus tumor-targeting abilities.^{4,5} They have been used in combination with either chemotherapy, radiation, or targeted therapies to improve antitumor efficacy.^{6,7}

However, the true antitumor potential of OVs is limited by a number of host-derived factors, including viral neutralization by preexisting antibodies, sequestration by the reticuloendothelial system, inadequate intravenous tumor delivery, and limited intratumoral virus replication and spread.^{8,9} Among the recognized factors that limit viral entry into tumor tissues, the tumor endothelium represents a barrier to the efficient delivery of viral and nonviral therapeutic agents into tumor cells after systemic administration.^{4,10} Therefore, the development of oncolytic agents that target the

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tumor vasculature may be one way to circumvent the above obstacles and improve viral entry into tumor tissues, leading to improved antitumor activity.

Mechanisms of tumor neovascularization

When a tumor reaches a diameter of ~ 2 mm³, it requires an independent blood supply to allow further growth.¹¹ The mechanisms by which tumors induce new blood vessel formation include angiogenesis (new vessel sprouting from pre-existing capillaries),¹² vasculogenesis (the formation of de novo capillaries from bone marrow-derived endothelial progenitor cells),¹³ vessel cooption,¹⁴ and vasculogenic mimicry.¹⁵

Tumor angiogenesis is regulated by a fine balance between endogenous pro- and antiangiogenic factors present in the tumor microenvironment (Figure 1). The expression of pro-angiogenic factors (vascular endothelial growth factor [VEGF], basic fibroblast growth factor [bFGF], platelet-derived growth factor [PDGF], epidermal growth factor [EGF], interleukin 8 [IL-8], the angiopoietins)¹⁶ by tumor or stromal cells is regulated by factors such as hypoxia, oncogene activation, or tumor suppressor silencing.¹⁷ Endogenous inhibitors of

angiogenesis include thrombospondin-1,¹⁸ as well as peptides derived from plasma (angiostatin)¹⁹ or tumor stroma, such as endostatin, tumstatin, and canstatin,²⁰ among others. When the balance favors pro-angiogenic factors, the “angiogenic switch” is “turned on”, leading to endothelial cell activation, proliferation, migration, matrix degradation, and capillary formation.²¹ The resulting tumor neovessels are structurally and functionally different from normal blood vessels, as they are leaky, tortuous, and disorganized. Tumor endothelial cells have aberrant morphology, lack pericytes, and have an abnormal basement membrane. These structural differences lead to an abnormal tumor microenvironment, hypoxia, acidosis, and an elevated tumor interstitial pressure, which contributes to impaired delivery of chemotherapy agents and resistance to standard therapies.²²

The understanding of the above mechanisms has led to the development and FDA approval of agents that target angiogenesis pathways. Most of the clinically available angiogenesis inhibitors target the VEGF pathway, either by targeting the ligand (bevacizumab, aflibercept)^{23,24} or the receptor (ramucirumab, sorafenib, sunitinib, pazopanib, axitinib, regorafenib).^{25–28}

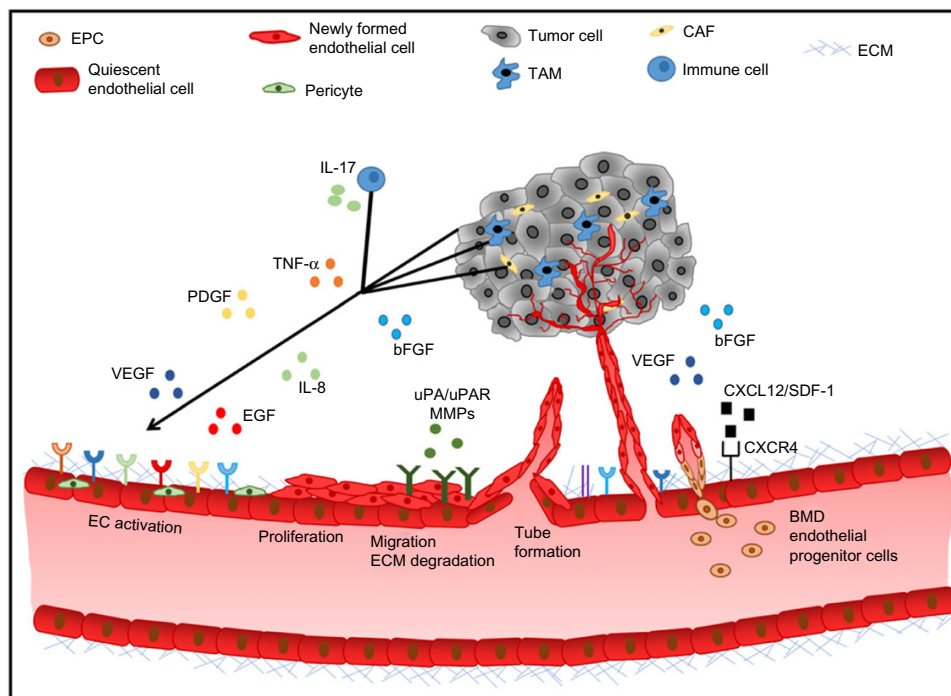


Figure 1 Tumor angiogenic cascade.

Notes: Quiescent endothelial cells become activated when stromal, tumor, and immune cell-derived pro-angiogenic factors are secreted into the tumor site leading to proliferation migration, ECM degradation, and tube formation from existing capillaries. BMD EPCs are recruited to the tumor site and differentiate into endothelial cells, forming de novo capillaries (vasculogenesis). The resulting tumor blood vessels are morphologically and functionally distinct from normal vasculature.

Abbreviations: bFGF, basic fibroblast growth factor; BMD, bone marrow-derived; CAF, cancer associated fibroblasts; EC, endothelial cell; ECM, extracellular matrix; EGF, epidermal growth factor; EPC, endothelial progenitor cell; IL-8, interleukin 8; IL-17, interleukin 17; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; SDF-1, stromal cell-derived factor 1; TAM, tumor-associated macrophages; TNF- α , tumor necrosis factor alpha; uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; VEGF, vascular endothelial growth factor.

Antiangiogenic therapies, however, have several limitations. One is related to side effects.²⁹ Clinically important toxicities from these agents include vascular-related side effects, such as hypertension, thromboembolic, or bleeding events,³⁰ which may be potentially severe, requiring close follow-up of patients treated with these drugs. Another limitation relates to the fact that these agents are cytostatic, and not cytotoxic; therefore, antiangiogenic agents are not curative.²⁹ Moreover, even though these agents provide initial clinical benefit, the majority of patients eventually progress due to the development of acquired resistance.^{31,32} Mechanisms of resistance to antiangiogenic agents are reviewed elsewhere.³³

Advances in the knowledge of tumor angiogenesis have allowed the development of OV's with the ability to target tumor vasculature. Of the ~2,600 papers published on oncolytic virotherapy between 2005 and 2015, ~5% of them focus on vascular targeting. The distinctive characteristics between normal and tumor vasculature have enabled scientists to develop several vascular-directed oncolytic viral strategies. Such strategies include: 1) unmodified OV's that have an endogenous ability to bind tumor blood vessels, 2) targeting tumor endothelial cell surface receptors by viral engineering (targeted viruses), 3) transcriptional targeting of the tumor vasculature, or 4) by the delivery of peptides/cytokines that inhibit angiogenesis by "armed" viral vectors. In addition, a number of studies have combined vascular-targeted viruses with other antiangiogenic or antitumor strategies, demonstrating enhanced efficacy.

Here, we provide an update on the strategies toward the design of oncolytic viral vectors with the ability to affect tumor vasculature, focusing on the main OV platforms that have been reported to target tumor neovascularization.

Oncolytic viral platforms associated with vascular targeting or antivascular activity

Adenovirus and adeno-associated virus

Wild-type adenoviruses bind coxsackie virus-adenovirus receptor (CAR) and internalize using integrin receptors (avb3, avb5).³⁴ Since CAR expression is highly variable in cancer and normal cells, adenoviruses require modifications in their genomes to increase tumor selectivity.³⁵ Currently, there are ~80 cancer clinical trials using adenoviruses as a platform, most of which are in Phases I and II.

Adenoviral vectors can be directed to tumor vasculature by either transcriptional or transductional retargeting (Table 1). VB-111 is a nonreplicating adenoviral vector (Ad-5, E1 deleted), containing a modified murine pre-proendothelin

promoter (PPE-1-3X) and a Fas-chimera transgene (Fas and human TNF receptor 1). VB-111, which is undergoing early clinical evaluation,³⁶ infects angiogenic vasculature, leading to improved antitumor effects in xenograft and syngeneic cancer models.³⁷ Ad5ROBO4 is an E1- and E3-deleted adenovirus containing the endothelial human roundabout4 (ROBO4) enhancer/promoter, enabling the vector to target tumor endothelial cells.³⁸

FGF2-Ad-TK, an adenovirus retargeted to FGF2 that expresses the herpes simplex virus thymidine kinase (HSV-*tk*) reduces tumor microvessel density (MVD), induces apoptosis and antitumor effects in vivo.^{39,40} Other adenoviral vectors, designed to target tumor endothelium via endothelial selectins⁴¹ or CD46,⁴² show important in vivo antitumor and antiangiogenic effects. KOX/PEGbPHF is an adenoviral vector coated with a pH-sensitive block copolymer, expressing a VEGF promoter-targeting transcriptional repressor (KOX), which targets the acidic tumor microenvironment and inhibits tumor growth and angiogenesis in vitro and in vivo.⁴³

Armed adenoviruses

A large number of "armed" adenoviruses have been designed to suppress angiogenic factors, especially VEGF. This has been achieved by either introducing shRNAs against VEGF,⁴⁴ soluble VEGF receptors,⁴⁵⁻⁴⁸ or VEGF promoter-targeted artificial zinc-finger proteins.⁴⁹ Ad-uPAR-MMP-9 is a replication-deficient adenovirus expressing antisense urokinase receptor (uPAR) and antisense Matrix metalloproteinase (MMP)-9, and therefore inhibits the expression of these important angiogenic targets in tumor tissues.⁵⁰ Other adenoviral vectors exert antiangiogenic effects in vitro and in vivo by expressing antiangiogenic molecules, including endostatin,⁵¹ angiostatin,⁵² or an endostatin/angiostatin fusion.⁵³ Armed adenoviruses can reach the tumor vasculature via circulating endothelial progenitor (CEP) cells. Infection of CEPs ex vivo with an adenovirus expressing soluble CD-115 resulted in significant antitumor effects and inhibition of tumor neovascularization in prostate cancer xenografts.⁵⁴

Adeno-associated viral vectors have been designed to target angiogenesis by expressing bevacizumab (AAVrh10. BevMab),⁵⁵ endostatin, thrombospondin-1,⁵⁶ or plasminogen kringle 5.⁵⁷ These agents have shown successful induction of antiangiogenic and antitumor effects in vivo.

Combination strategies

Bevacizumab, an anti-VEGF monoclonal antibody, given before treatment with CRAAd-S-pk7, a conditionally replicating adenovirus with selectivity to glioma cells, induces MMP-2

Table 1 Adenoviral vectors

Virus name	Mechanism	In vitro	In vivo	Delivery route	Reference
Retargeted					
FGF2-Ad-TK	FGF2 receptor targeting by a conjugate of FGF2 linked to a Fab' fragment against the adenoviral knob region	N/A	↓ Tumor MVD and antitumor effects in HNSCC xenografts	IT	40
MHESpcAdluc	Polymer-coated Ad linked to monoclonal antibodies against human E-selectin	Infects umbilical cord ex vivo	N/A	N/A	41
PSGL-1-Fc-StrepGpcAdluc (in vivo)	Polymer-coated Ad linked to PSGL-1	N/A	Co-localizes with CD31+ cells in HCC xenografts	IV	
Ad-uPAR-MMP-9	Replication-deficient Ad expressing uPAR and MMP-9 antisense transcripts	↓ Cancer cell-induced EC capillary formation	↓ Tumor growth and lung metastasis in NSCLC xenografts	IT, IV	50
KOX/PEGbPHF	pH-sensitive oncolytic adenovirus expressing a VEGF transcriptional repressor	↓ VEGF expression ↓ Vessel sprouting	↓ MVD and antitumor effects in rat glioma	IV	43
Ad5/35LacZ	CD 46 dependent endothelial binding	Efficient infection of endothelial cells	Rat HCC model: Colocalization of viral β-gal with CD46, CD31, Flk1	Via hepatic artery	42
VB-111	Ad-5 containing a pre-proendothelin 1 promoter (PPE-1-3X) and Fas-chimera transgene	N/A	Transgene expression and apoptosis in tumor-associated ECs	IV	37
Ad5ROBO4	Replication-competent Ad5 containing ROBO4 promoter	N/A	RCC model: Tumor EC infection ↓ MVD	IV	38
Armed					
Ad5H2E-PPE1(3x)-ASMase	Endothelial targeted expression of acid sphingomyelinase	Specificity toward ECs	Melanoma; fibrosarcoma: Tumor EC-specific targeting ↑ EC apoptosis	IV	112
Ad-ΔB7-shVEGF	VEGF-specific shRNA expressing Ad vector	↓ Tube formation ↓ Vessel sprouting (rat aortic ring assay)	Matrigel plug assay ↓ Vascularization ↓ Tumor growth in multiple tumor models	IT	44
Ad-ΔE1-U6shIL8	Replication-competent Ad expressing IL-8 shRNA	↓ Migration and tube formation ↓ Vessel sprouting (rat aortic ring assay)	Breast cancer lung metastasis model; ↓ VEGF and IL-8 ↓ Tumor MVD	IT	113
CRAAd-S-5/3shMMP14	Fiber-modified Ad vector expressing MMP14 shRNA	N/A	Glioma xenografts ↓ CD31+ cells	IC	114
Ad5/3-9HIF-Δ24-VEGFR-1-Ig	Hypoxia targeted Ad vector expressing soluble VEGF receptor 1-Ig fusion protein	Minimal HUVEC cytotoxicity	RCC xenografts: ↓ Tumor size ↓ Tumor MVD	IT	45
Ad5/F35-XAF1	Expression of tumor suppressor gene XAF1	↓ VEGF mRNA and protein expression	HCC xenografts: ↓ Tumor VEGF expression ↓ Tumor MVD	IT	115
ZD55-IL-18	E1B gene deleted Ad expressing IL-18	N/A	Melanoma xenografts: ↓ VEGF ↓ CD34 positive cells	IT	116
EndoAngio-PRRA	Prostate-restricted Ad5 expressing Endostatin-angiostatin fusion protein	Conditioned media ↓ EC proliferation, migration, and tube formation	Prostate cancer xenografts: antitumor effects ↓ Tumor MVD	IT	53
pAd-2S03	Ad expressing a scAb intrabody that inhibits mouse and human Tie-2 surface expression	Effective intrabody expression in ER of ECs	Human Kaposi's sarcoma and colon carcinoma ↓ Tumor MVD	Peritumorally	117

(Continued)

Table 1 (Continued)

Virus name	Mechanism	In vitro	In vivo	Delivery route	Reference
Ad-eTieI-GALV	Ad encoding GALV fusogenic membrane glycoprotein regulated by human endothelial receptor tyrosine kinase (eTieI)	Heterocellular syncytia between tumor-associated endothelial cells and tumor cells	HEK 293 xenografts: heterocellular syncytia between infected ECs and HEK 293 cells lead to virus replication in vivo	IV	118
Combination					
Ad Flk1-Fc + DI922/947	Soluble VEGFR2 expressing Ad vector	N/A	Prostate and colon cancer xenografts: ↑ Antitumor effects ↓ Tumor MVD	IT IV	47
Ad-Endo + Ad-H101	Replication-deficient Ad encoding human endostatin + replication-competent Ad	↓ ECs proliferation	NPC xenografts: combined treatment ↑ Endostatin expression ↑ Antitumor effects	IT	51
dI922-947 + bevacizumab	E1 gene deleted Ad + anti-VEGF monoclonal antibody	N/A	Thyroid carcinoma; ↑ Tumor viral distribution ↓ MVD	IT	119
AdVIL-24 + ionizing radiation	Ad5E1- and E3-deleted adenovirus expressing human IL-24 and GFP	N/A	NPC xenograft models: ↓ Tumor MVD ↓ VEGF expression ↑ Antitumor effects	IT	59

Abbreviations: Ad, adenovirus; β -gal, β -galactosidase; EC, endothelial cell; ER, endoplasmic reticulum; FGF, fibroblast growth factor; GALV, gibbon ape leukemia virus; HEK, human embryonic kidney cells; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; IC, intracranial; IL, interleukin; IT, intratumoral; IV, intravenous; MMP-9, matrix metalloproteinase 9; MVD, microvessel density; N/A, not applicable; NPC, nasopharyngeal carcinoma; NSCLC, non-small-cell lung cancer; PPE-1-3X, pre-endothelin promoter; PSGL, P-selectin glycoprotein ligand-1-Fc fusion; RCC, renal cell cancer; ROBO4, roundabout4; scAb, single chain antibody; shRNA, short hairpin RNA; uPAR, urokinase receptor; HUVEC, human umbilical vein endothelial cell; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; ↑, increased; ↓, decreased.

activity, extracellular matrix degradation, and increased intratumoral viral distribution.⁵⁸ AdVIL-24, an E1- and E3-deleted adenovirus expressing both human IL-24 and green fluorescent protein (GFP), in combination with ionizing radiation, was associated with decreased tumor VEGF expression, decreased microvessel density, and in vivo antitumor effects in a nasopharyngeal carcinoma.⁵⁹ Other combination strategies are presented in Table 1.

Herpes simplex virus

The majority of Herpes simplex virus type 1 (HSV-1) vectors used for oncolytic virotherapy are replication-competent with genome modifications. For example, deletion of both the copies of γ 34.5 gene is commonly performed to reduce neurovirulence. The gene product of γ 34.5, ICP34.5, directs protein phosphatase 1 to specifically dephosphorylate eIF2 α , leading to inhibition of the protein synthesis shutoff.⁶⁰ ICP6 gene encodes for the large subunit of ribonucleotide reductase, and it is needed to replicate in nondividing neurons. Inactivation of these genes allows efficient tumor cell specificity as it will only replicate in dividing cells.⁶¹

There are ~18 clinical trials using HSV in cancer patients, with some vectors in advanced stages of clinical development.⁶²

There is no clear consensus as to whether unmodified HSV-1 vectors have endogenous vascular binding abilities. While some studies have shown that oncolytic HSV-1 has a truly innate ability to infect murine and human endothelial cells in vitro and in vivo,^{63,64} other reports suggest that HSV-1 may actually elicit a potent angiogenic response.⁶⁵⁻⁶⁷ Most of the recombinant “antiangiogenic” HSV-1 vectors are armed viruses targeting pro-angiogenic factors or expressing angiogenesis inhibitors (Table 2).

Armed HSV vectors and combination strategies

The great majority of in vivo experiments using armed oncolytic HSVs have used the intratumoral route of administration. Treatment with T-TSP-1, an HSV-1 vector expressing thrombospondin-1 is associated with reduced tumor MVD and improved antitumor effects.⁶⁸ bG47 Δ PF4 induces antiangiogenic effects in vitro and in vivo by expression of soluble platelet factor-4 (PF4), in models of glioblastoma and peripheral nerve sheet tumors.⁶⁹ Other armed oncolytic HSV vectors have shown antiangiogenic and antitumor effects in vitro and in vivo by the expression of vasculostatin,⁷⁰ TIMP-3,⁷¹ angiostatin, endostatin,^{72,73} or IL-12.⁷⁴

Table 2 Herpes simplex virus

Virus	Mechanism	In vitro	In vivo	Delivery route	Reference
Recombinant					
G207	HSV-1 gene disruption of ICP6 + LacZ gene	ECs sensitive to replicative and cytotoxic effects Disrupts endothelial cell tubes	Matrigel plug assay: ↓ Vessel perfusion Rhabdomyosarcoma: ↓ Tumor vasculature	IT	120
Armed					
RAMBO	Expression of vasculostatin under the control of IE4/5	↓ EC migration ↓ Tube formation	Glioma xenografts: ↓ Reduction in tumor vascular volume fraction and MVD	IT	70
bG47Δ-PF4	Expression of soluble platelet factor-4	↓ Migration ↑ EC apoptosis	Glioma xenografts Murine MPNSTs: ↓ Tumor growth and ↓ MVD	IT	69
rQT3	HSV-1 expressing TIMP-3	Efficient EC replication and cytotoxic effects	Neuroblastoma xenografts: MPNST xenografts ↓ Tumor growth and MVD ↓ CEP recruitment to tumor site	IT	71
NV1042	HSV carrying the murine IL-12 gene	CM of virus infected SCCs ↓ ECs tube formation	Murine SCC model: ↓ Tumor growth ↓ Tumor MVD ↑ Levels of IFN-γ and IFN-inducible protein 10	IT	121
T-TSP-1	HSV expressing human TSP-1	N/A	Gastric cancer xenografts: ↓ CD31 staining in tumors	IT	68
VAE	HSV F expressing Endo-Angio fusion protein	N/A	Glioma xenografts; ↓ Tumor MVD ↓ Tumor growth	IT	73
Combination					
G47Δ-mAngio + G47Δ-mIL12	G47Δ viruses expressing murine angiostatin and IL-12	↓ EC tube formation	Glioma xenografts: ↑ Tumor virus distribution ↓ Tumor VEGF ↓ Tumor MVD	IT	74
G207 + hrR3 + erlotinib	HSV-1 mutant vectors EGFR TKI	EC cytotoxicity and viral replication	MPNST xenografts: Tumor EC targeting ↓ Pro-angiogenic gene expression ↓ Tumor MVD ↑ Antitumor effects	IT	76
cRGD + HrR3	Angiostatic peptide Mutant HSV-1 vector	N/A	Rat glioma: ↓ Tumor vascular permeability ↑ Survival	IT	78
HF10 + bevacizumab	Mutant HSV-1 strain HF Anti-VEGF monoclonal Ab	N/A	Breast cancer xenografts: ↑ Tumor virus distribution ↓ MVD ↑ Antitumor effects	IT	77
NV1042 + vinblastine	oHSV secreting IL-12+ MDA	↓ EC tube formation	Prostate cancer xenografts; ↓ CD31 staining in tumors ↓ Tumor burden	IT	75

Abbreviations: Angio, angiostatin; CEP, circulating endothelial progenitor; ECs, endothelial cells; MPNSTs, malignant peripheral nerve sheath tumors; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; Endo, endostatin; HSV-1, Herpes simplex virus type 1; IFN, interferon; IL, interleukin; IT, intratumoral; MDA, microtubule disrupting agent; MVD, microvessel density; N/A, not applicable; PF4, platelet factor-4; TIMP-3, tissue inhibitor of metalloproteinases; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; ↑, increased; ↓, decreased.

Zhang et al demonstrated that combined treatment with recombinant HSV-1 vectors carrying murine angiostatin (G47Δ-mAngio) and IL-12 (G47Δ-mIL12) on a human glioma xenograft model was associated with improved survival compared to treatment with each individual virus.⁷⁴ Combination of NV1042, an oncolytic HSV expressing

IL-12, and vinblastine has superior antiangiogenic and anti-tumor effects in human prostate cancer xenograft model, when compared to the parental virus alone, or the parent virus and vinblastine.⁷⁵ Oncolytic HSVs have been successfully combined with agents like erlotinib,⁷⁶ bevacizumab,⁷⁷ and RGD (arginylglycylaspartic acid) peptides⁷⁸ in models

Table 3 Vaccinia virus

Virus	Mechanism	In vitro	In vivo effects	Delivery route	Reference
Armed					
wDD-SR-RFP	VV expressing human somatostatin receptor subtype 2 and RFP	↑ Viral replication after VEGF stimulation	Colon cancer xenografts Colocalization of the viral RFP within CD31+ cells ↓ CD31 staining	IP	122
JX-795	VV <i>B18R</i> deletion mutant expressing IFN-β	N/A	Murine mammary carcinoma: ↓ Tumor perfusion ↑ Antitumor effects In vivo tumor ECs targeting	IV; IT	86
GLV-1h446	Expression of scAb against VEGF and FAP	N/A	Feline mammary carcinoma; ↓ Tumor growth ↓ Tumoral VEGF levels and angiogenesis	IV	83
wdd-VEGFR-I-Ig	Expression of soluble VEGFR I	N/A	RCC (human and murine) ↑ Antitumor effects ↓ MVD	IV	84
OVV-CXCR4-A-mFc	Expression of CXCR4 antagonist	N/A	Murine breast cancer; ↓ Tumor perfusion ↓ Tumor CXCL 12, VEGF ↓ BMD endothelial and myeloid cells	IV	88
JX-594	hGM-CSF expressing VV, targeted toward cells with Ras/MAPK activation	Efficient EC replications EC cytotoxicity	Murine breast and colon cancer: Selective tumor EC targeting ↑ Antivascular cytokines Disrupts vasculature	IV	81
VVhEA	LV strain of VV expressing the Endo-Angio fusion gene	↓ EC tube formation	Pancreatic cancer xenografts: ↓ Tumor MVD ↑ Antitumor effects	IV; IT	85
Combination					
wDD-luc + Ad Flk1-Fc or wDD-luc + sunitinib	wDD-luc Ad expressing Flk1-Fc Anti-VEGFR TKI	wDD alone Inhibits ECs proliferation	Murine breast cancer; Enhancement of antitumor effects and improved survival with combination treatments	IV	82
JX-594 + sorafenib	VV + VEGFR/RAF TKI	N/A	Human HCC; murine melanoma; Combination improves antitumor efficacy	IV	89
VVDD-EGFP + PDT	TK and VGF-deleted VV expressing EGFP + PDT	N/A	Murine neuroblastoma, human SCC: PDT enhances viral replication ↑ Antitumor effects	IV	90

Abbreviations: RFP, red fluorescent protein; Angio, angiostatin; Ad, adenovirus; BMD, bone marrow-derived; ECs, endothelial cells; Endo, endostatin; HCC, hepatocellular carcinoma; hGM-CSF, human granulocyte-macrophage colony-stimulating factor; IFN-β, Interferon beta; FAP, fibroblast activation protein; IP, intraperitoneal; IT, intratumoral; IV, intravenous; LV, Lister vaccine; MVD, microvessel density; N/A, not applicable; PDT, photodynamic therapy; RCC, renal cell cancer; MAPK, mitogen-activated protein kinase; scAb, single chain antibody; SCC, squamous cell carcinoma; TK, thymidine kinase; VEGF, vascular endothelial growth factor; VGF, vaccinia growth factor; VV, vaccinia virus; VEGFR, VEGF receptor; EGFP, enhanced green fluorescent protein; ↑, increased; ↓, decreased.

of malignant peripheral nerve sheet tumors, breast cancer, and gliomas, respectively.

Vaccinia virus

Currently, there are ~56 clinical trials using vaccinia virus (VV), some of them showing promising results.⁷⁹ The biology and pathogenesis of this viral vector has been extensively characterized.⁸⁰ VV is known to intrinsically target tumor vasculature and induce vascular collapse after intravenous administration.⁸¹ Another proposed antiangiogenic

mechanism include VEGF downregulation during active viral infection.⁸²

JX-594 is an oncolytic VV (OVV) engineered to target cells with Ras/MAPK activation and to express the human granulocyte-monocyte colony-stimulating factor (hGM-CSF) and β-galactosidase (β-gal) transgenes. In vivo, JX-594 replicates in tumor-associated endothelial cells, leading to disruption of tumor blood flow and hypoxia, while normal vessels are not affected.⁸¹ In early phase trials, JX-594 showed satisfactory tolerability, viral replication, transgene

expression, and importantly, antivasular effects, as evidenced by disruption of tumor perfusion in patients with hepatocellular carcinoma.⁸¹

Armed VV vectors and combination strategies

Armed OVVs can target the VEGF pathway, either by expressing single chain antibodies against VEGF⁸³ or soluble VEGF receptor 1.⁸⁴ Other vectors express endostatin-angiostatin fusion protein,⁸⁵ or interferon beta.⁸⁶ All of the above viruses induce antiangiogenic and antitumor effects in vivo after intravenous administration (Table 3). CXCL12 and its receptor CXCR4 is a chemokine system that has been associated with angiogenesis, vasculogenesis, and tumor progression.⁸⁷ OVV-CXCR4-A-Fc is an OVV that delivers a CXCR4 antagonist expressed in the context of the murine Fc fragment of IgG2a. Intravenous administration of this viral vector resulted in inhibition of tumor growth and vascular disruption in murine mammary cancer, effects associated with decreased levels of CXCL12, VEGF, and circulating endothelial progenitor cells (CEPs).⁸⁸

JX-594 has been combined with sorafenib in murine cancer models and in patients with hepatocellular carcinoma. The combination was well tolerated and associated with decreased tumor perfusion and objective responses.⁸⁹ Combination of OVV with either adenoviral vectors expressing FLK1 Fc or Sunitinib was associated with improved antitumor effects in models of murine mammary cancer in vivo.⁸² Gil et al combined OVV with photodynamic therapy (PDT), showing that vascular disruption caused by PDT led to higher viral titers and improved antitumor in murine models of neuroblastoma and squamous cell carcinoma.⁹⁰

Vesicular stomatitis virus

Vesicular stomatitis virus (VSV) is a negative-stranded RNA virus that induces potent and rapid in vitro and in vivo antitumor effects.⁹¹ Currently, there is one Phase I clinical trial using VSV as an oncolytic vector in patients with hepatocellular carcinoma. The oncolytic ability of VSV is based on the knowledge that most cancer cells possess an impaired antiviral response induced by type I interferon, making them more susceptible to VSV infection than normal cells.⁹² The low density lipoprotein receptor has been recognized as the major cell surface receptor for VSV in human and mouse cells.⁹³

Oncolytic VSV has been shown to directly bind to tumor vasculature, reduce vascular perfusion due to clot formation, and decrease microvessel density.⁹⁴ Attempts

have been made to design oncolytic VSVs displaying endothelial targeting peptides, such as echistatin or RGD peptides. However, endothelial infection in vivo could not be demonstrated in tumors treated by the targeted viruses.⁹⁵ Studies combining oncolytic VSV and other vascular targeted agents have shown enhanced antitumor effects (Table 4).

Combination strategies

Combination of intravenous VSVΔ51 with the vascular disrupting agent ZD6126 or with radiation therapy demonstrated enhanced antitumor effects, compared to each agent alone.⁹⁶ ZD6126 increased viral delivery via vascular disruption and decreased interstitial fluid pressure. Sunitinib in combination with oncolytic VSV are associated with significant antitumor effects in models of prostate, breast, and kidney cancer, compared to each agent alone.⁹⁷ Finally, in a hepatocellular carcinoma model, combination of embolization and rVSV-F, a recombinant VSV expressing the Newcastle Disease Virus fusion protein, resulted in decreased tumor MVD, improved antitumor effects, and improved survival.⁹⁸

Measles virus

Measles virus (MV) is a negative-stranded RNA virus that belongs to the family of Paramyxoviridae.⁹⁹ The Edmonston vaccine strain of MV (MV-Edm) has oncoselectivity and promising antitumor activity in vitro and in mouse xenograft models. Currently, there are approximately seven active clinical trials using MVs as oncolytic vectors showing satisfactory results in terms of safety and promising antitumor effects.^{100,101} Three endogenous MV receptors have been identified: CD46 (ubiquitously expressed in cells), SLAM (expressed on immune cells),¹⁰² and Nectin-4. Nectin-4 is considered the epithelial receptor for this viral agent.¹⁰³

Targeted and armed oncolytic MV vectors

Oncolytic MV vectors have been engineered to target vasculature by displaying vascular-targeted ligands as C-terminal extensions of the MV-H protein (Table 5). The first reported vascular-targeted oncolytic MV is MV-ERV, which displays echistatin, a disintegrin that binds with high affinity to integrin $\alpha v \beta 3$.¹⁰⁴ This agent was shown to bind and infect endothelial cells in vitro and vasculature in vivo and induced potent antitumor effects.¹⁰⁵ An MV vector displaying RGD peptides able to bind endothelial cells via $\alpha v \beta 3$ and $\alpha 5 \beta 1$ (MV-RGD) was shown to target neovessels in the ear pinna angiogenesis model.¹⁰⁶

Table 4 Vesicular stomatitis virus

Virus	Virus design	In vitro	In vivo effects	Delivery route	Reference
Recombinant					
VSV-GFP	Δ51 VSV-expressing GFP HR strain of wt Indiana virus	N/A	Murine colon cancer; Infects and induces clot formation in tumor vasculature ↓ Tumor perfusion ↓ MVD	IV	94
Combination					
VSVΔ51 + ZD6126	Mutant VSV ZD6126: vascular disrupting agent	N/A	Human HNSCC: Combination therapy: ↑ Viral replication ↑ Antitumor effects	IV	96
rVSV-F + DSM	VSV-expressing NDV fusion protein + embolization with degradable starch microspheres	N/A	Rat HCC; ↑ Tumor necrosis ↑ Apoptosis ↓ MVD (CD 31)	Via hepatic artery	98
VSV (M51R) + Sunitinib	Attenuated mutant VSV Anti-VEGFR TKI	N/A	Prostate, breast, and kidney tumors Increased antitumor effects of the combination	IT	97

Abbreviations: DSM, degradable starch microspheres; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; HR, heat resistant; IV, intravenous; MVD, microvessel density; N/A, not applicable; NDV, New castle disease virus; VEGFR, vascular endothelial growth factor receptor; VSV, vesicular stomatitis virus; wt, wild-type; ↑, increased; ↓, decreased.

MV-uPA is a fully retargeted oncolytic MV directed against the uPAR. This vector was generated by displaying the aminoterminal fragment of human or mouse urokinase into the C-terminus of a mutant MV-H unable to bind to CD46 or SLAM.¹⁰⁷ In vitro, MV-human-uPA efficiently infected and replicated in human umbilical vein endothelial cells stimulated

with VEGF. MV-mouse-uPA was able to infect murine tumor vasculature, as evidenced by MV-N and CD31 colocalization in tumor tissues.¹⁰⁷ Both retargeted viruses induce species-specific antitumor and antimetastatic effects.^{108,109}

MV-E:A encodes human or murine endostatin/angiostatin fusion protein (MV-hE:A and MV-mE:A,

Table 5 Measles virus

Virus	Mechanism	In vitro	In vivo	Delivery route	Reference
Targeted					
MV-ERV	MV displaying echistatin	Infects and ↓ EC tube formation Infects newly formed blood vessels in chorioallantoic membrane assays	Multiple myeloma tumors; ↓ Tumor volume	IT	105
MV-RGD MV-echistatin	MV displaying RGD and echistatin peptides as C-terminal extensions of MV-H protein	N/A	Infection of neovessels in ear pinna angiogenesis model and CAM assays Myeloma xenografts: MV-N colocalizes with CD31+ cells	IV	106
MV-h-uPA MV-m-PA	MV-Edm fully retargeted against human or murine uPAR	Efficiently infects and replicates in VEGF stimulated ECs Infects endothelial capillaries	Breast cancer MV-N colocalizes with CD31+ cells	IV	107
Armed					
MV-E:A	Expression of Angio and Endo	CM of infected cancer cells ↓ EC tube formation ↓ ECs migration	Medulloblastoma xenografts: ↓ Tumor microvessels	IT	110
MV-mIFN-β	Expression of murine IFN-β	N/A	Mesothelioma xenografts: ↓ Tumor MVD (CD31)	IT	111

Abbreviations: Angio, angiostatin; EC, endothelial cell; Endo, endostatin; IFN-β, interferon beta; IT, intratumoral; IV, intravenous; MV, measles virus; MVD, microvessel density; N/A, not applicable; CAM, chick chorioallantoic membrane; uPA, urokinase plasminogen activator; uPAR, urokinase receptor; VEGF, vascular endothelial growth factor; ↓, decreased.

respectively).¹¹⁰ In vivo, intratumoral injection of the recombinant viruses in a medulloblastoma xenograft model was associated with decreased MVD. MV-mIFN β is a murine interferon beta expressing MV, which was found to decrease microvessel density (measured by CD31 expression) after intratumoral administration in malignant mesothelioma.¹¹¹

Conclusion and future directions

Recent studies have shown proof of concept that vascular targeting by OV's is a feasible and promising strategy, associated with significant antiangiogenic and antitumor effects. Angiogenic pathways previously thought to be targetable only by small molecules or antibodies can now be targeted by redesigned OV's. This strategy is unique and offers an advantage over current antiangiogenic agents. In addition to targeting tumor vasculature, OV's exert potent oncolytic and immunomodulatory effects, which may help overcome tumor-resistance mechanisms.

However, there are challenges to the clinical development of vascular-targeted viruses. One of the main translational questions involves the safety of vascular targeting by an OV. As animal studies are not always predictive of the clinical scenario, it is extremely important that preclinical and clinical testing of "antiangiogenic" OV's take into account the potential for toxicity to normal vasculature. Experience from currently approved antiangiogenic agents shows that "tumor endothelial" targeted agents are associated with significant off-target effects in normal vasculature. Therefore, extensive preclinical toxicity studies will be required, focusing on the virus effects on vasculature, before moving such agents into the clinic. Clinical trials of such agents will require careful planning in regard to trial design, dosing schedule, patient selection, and methods to monitor the OV's' safety and biological effects. This can be achieved by multidisciplinary discussion among scientists, clinical investigators, ethics committees, and regulatory agencies.

Finally, combination studies using vascular-targeted viruses and standard/targeted therapies should be carefully evaluated in appropriate preclinical models that closely resemble human cancers. This will ensure safe and effective translation of this highly attractive strategy into a novel clinical therapeutic option.

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Disclosure

The authors report no conflicts of interest in this work.

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