

# Update on oncolytic viral therapy – targeting angiogenesis

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**Abstract:** Oncolytic viruses (OVs) have the ability to selectively replicate in and lyse cancer cells. Angiogenesis is an essential requirement for tumor growth. Like OV, the therapeutic effect of many angiogenesis inhibitors has been limited, leading to the development of more effective approaches to combine antiangiogenic therapy with OV. Angiogenesis can be targeted either directly by OV infection of vascular endothelial cells, or by arming OV with antiangiogenic transgenes, which are subsequently expressed locally in the tumor microenvironment. In this review, we describe the development and targeting of OV, the role of angiogenesis in cancer, and the progress made in arming viruses with antiangiogenic transgenes. Future developments required to optimize this approach are addressed.

**Keywords:** oncolytic virotherapy, cancer

## Introduction to oncolytic viral therapy

Cancer remains a leading cause of death despite incremental advances in surgery, chemotherapy, and radiotherapy. This has led to the development of new treatment strategies. With increasing knowledge of the genetic defects and molecular basis of cancer, gene therapy has become an attractive option, although clinical trials to date have shown only moderate efficacy. Since cancer usually results from a combination of genetic defects, strategies to eradicate cancer cells themselves are likely to have a greater efficacy than attempts to correct each genetic defect individually. The ideal vector has tumor-selective oncolytic properties in that, as well as delivering gene therapy, it can selectively enter and destroy cancer cells, without affecting surrounding normal cells. It should also be safe to administer, have minimal side effects, be easy to mass-produce and purify, and be genetically stable in storage and use.

While nonviral vectors, including naked DNA or DNA packaged in liposomes and dendrimers, have been used in cancer gene therapy,<sup>1</sup> it is viruses that have been recognized to be the most efficient means currently available for the delivery of therapeutic genes. The evolution of viruses over millions of years has resulted in their ability to evade our immune system, infect, and replicate efficiently in human cells before causing their death to facilitate viral spread.<sup>2</sup> While the viral vectors developed initially were nonreplicating, it is the ability of viruses to replicate, so amplifying a small input dose to maximize therapeutic effect, that really sets them apart from other vectors. Interest has therefore shifted to the development of oncolytic viruses (OVs). These are replication-selective in that they have the ability to replicate in and cause death of tumor cells, while sparing normal cells.<sup>3</sup> This property may be inherent or

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enhanced through genetic engineering and the viruses armed to deliver therapeutic transgenes, such as angiogenesis inhibitors.

The use of viruses for cancer treatment is not a new concept since, at the beginning of the 20th century, it was observed that a flu-like disease resulted in decreased tumor load in a patient with leukemia and that rabies vaccination was followed by regression of cervical cancer.<sup>4</sup> OV's can be engineered to express foreign genes and, therefore, deliver gene therapy on a continuous basis to augment their therapeutic effect. This combination of mechanisms means that resistance, which often limits standard chemotherapy, is less likely to occur.<sup>5</sup>

## Barriers to oncolytic viruses

Over the last two decades, thousands of OV's have been developed, resulting in many clinical trials but few examples of clinical efficacy.<sup>6</sup> Replication-selective oncolytic adenovirus is the most well-researched, and dl1520 (or H101 in the People's Republic of China) was approved as the world's first oncolytic virotherapy for the treatment of head and neck cancer.<sup>7</sup> This has also been administered by intratumoral injection into patients with locally advanced pancreatic tumors in Phase I/II trials.<sup>8</sup> Although treatments were well tolerated, efficacy was poor. The main barriers to oncolytic viral therapy have been recognized as viral attenuation caused by the genetic engineering to improve tumor selectivity, the effects of the tumor microenvironment, and the host immune response.<sup>9,10</sup>

## Tumor selectivity

The first generation of OV's were inherently tumor selective in their replication and included Newcastle virus<sup>11</sup> and reovirus.<sup>12,13</sup> Second generation OV's were engineered to limit replication to tumors by inserting tumor-specific promoters, such as the prostate-specific antigen (PSA), upstream of the genes required for viral replication.<sup>14</sup> The deletion of genes, such as thymidine kinase (TK), that are themselves required for viral replication in normal cells, but not in tumor cells also enhances tumor-selectivity. Many other strategies have been developed in order to increase the selectivity of OV's.<sup>10</sup> However, the efficiency of cell lysis and cell-to-cell transfer of OV's alone in vivo is often poor since these modifications can result in the attenuation of antitumor potency.

## Tumor microenvironment

The importance of the interaction between cancer cells and their environment is well recognized.<sup>15</sup> Tumor development has many similarities with wound healing, as in

tumor development, there is also a persistent inflammatory response, with many of the same cytokines released from the fibroblasts, which attract immune cells, including macrophages, which in turn, release further cytokines that promote angiogenesis.<sup>16</sup> Considering these complex interactions, it is not surprising that OV's targeting the tumors alone have had limited efficacy.

Collagen in the extracellular matrix (ECM) has been found to be a physical barrier to the spread of OV's within tumors. It has been shown that inducing collagen degradation with a bacterial collagenase improved the spread and efficacy of an oncolytic herpes simplex virus.<sup>17</sup> The construction of an oncolytic adenovirus expressing the collagenase matrix metalloproteinase (MMP)-9 also increased viral spread in human pancreatic and lung cancer models.<sup>18</sup> The inevitable hypoxic environment in most tumors has also been found to attenuate oncolytic adenoviral therapy.<sup>19</sup> OV's can also increase the vascular permeability in tumors, stimulating angiogenesis.<sup>20</sup> Antiangiogenic therapy may itself reduce the innate immune response by stabilizing the tumor vasculature and decreasing the immune cell infiltration of tumors.<sup>21</sup>

## Host immune response

The host immune response to virus-infected tumors may be the most significant limitation to oncolytic viral therapy. Soon after virus delivery to tumors, an innate immune response is observed, with recruitment of neutrophils, natural killer cells, and macrophages.<sup>22</sup> Inhibition of the innate immune response may enhance the efficacy of OV's.<sup>9</sup> However, the step-wise deletion of virus virulence genes that normally cause evasion of the host immune response has been used to improve OV tumor selectivity. The host immune response is a double-edged sword for OV-based therapeutics. On the one hand, a vigorous host immune response to the OV can result in rapid viral clearance before the virus is able to exert a therapeutic effect or even result in tumor progression due to immunosuppression. The efficacy of multiple injections of the same virus may be further limited by a neutralizing antibody response.<sup>23</sup> However, the host immune response may be critical to the efficacy of oncolytic viral therapy. This may be mediated via innate immune effectors, adaptive antiviral immune responses eliminating infected cells, or adaptive antitumor immune responses.<sup>24</sup>

## Choice of transgene expression by oncolytic viruses

The choice of genes possible for cancer gene therapy is vast. The main mechanisms of action of potential therapeutic

genes are those considered to be corrective, immunomodulatory, and cytoreductive, all of which have been delivered by OV<sub>s</sub>.

## Corrective genes

Unregulated cell growth in cancers is caused by mutations in oncogenes or tumor suppressor genes (TSGs). Therefore, inhibiting oncogenes or upregulating TSGs may restore normal cell growth and division. The most commonly targeted TSG is *p53*, as mutations of this gene are estimated to be found in over half of all malignancies.<sup>25,26</sup> In 2003, a recombinant, nonreplicating adenovirus expressing *p53* (rAd-53 or Gendicine<sup>TM</sup>; Schenzhen SiBiono Gene Tech Co, Ltd, Schenzhen, People's Republic of China) was approved by the State Food and Drug Administration of China for the treatment of head and neck squamous cell carcinoma and became the first licensed gene therapy product in the world. Another commonly mutated TSG is *p16*, which has been delivered by oncolytic adenovirus to treat gastric cancer xenografts.<sup>27</sup> However, since most cancers result from defects in several genes, this strategy has not been effective, and focus has moved to targeting signaling pathways.

## Immunomodulatory genes

As discussed previously, the host's innate immune response to viruses can lead to their rapid clearance thus limiting transgene expression and antitumor efficacy. The adaptive immune response may preclude repeated virus administration through the formation of circulating antibodies. Many malignant cells express tumor-associated antigens (TAAs). Recognition of TAAs by antigen-presenting cells leads to the activation of TAA-specific CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs). These in turn, cause tumor cell death through direct lysis. Tumors avoid CTL destruction by limiting TAA presentation and reducing the expression of major histocompatibility complex class I (MHC I), which is also required for antigen recognition.<sup>28</sup> Cancer gene therapy can enhance tumor cell recognition through increased TAA presentation or upregulated MHC I expression.<sup>29</sup> Cytokine delivery by gene therapy, such as oncolytic adenovirus- or vaccinia virus (VV)-delivered interferon  $\beta$ , has shown potential in modulating the host immune response to improve tumor clearance.<sup>30,31</sup> Granulocyte-macrophage colony-stimulating factor (GM-CSF)-armed OV<sub>s</sub> (herpes simplex virus [HSV] and VV) have shown encouraging results in clinical trials,<sup>32,33</sup> although tumor-derived GM-CSF has also been recently demonstrated to drive the progression of cancer.<sup>34,35</sup> The mechanism is thought to be similar in both situations, with GM-CSF

suppressing T cell immunity, allowing OV<sub>s</sub> and tumor cells, respectively, to evade the host immune response.

## Cytoreductive genes

Cytoreductive therapy targets cancer cells either directly or indirectly. Methods include gene-directed enzyme prodrug therapy (GDEPT) (also called suicide gene therapy) and antiangiogenic therapy. Acting in cancer cells only, GDEPT expresses a gene encoding an enzyme that converts a pro-drug into a potential cytotoxin. The cytotoxin is produced exclusively within the target tumor to cause tumor cell death and regression.<sup>36</sup> One example is an oncolytic adenovirus expressing a fusion protein that converts 5-fluorocytosine into the commonly used chemotherapeutic agent 5-fluorouracil.<sup>37</sup> Virus-delivered GDEPT has entered Phase I clinical trials.<sup>38,39</sup> The requirement of angiogenesis for the growth of all tumors has led to the discovery of a wide variety of angiogenesis inhibitors. This strategy will now be explored in detail.

## Introduction to angiogenesis

Angiogenesis is the growth of new capillary blood vessels from existing vessels. This is necessary for the growth of all tumors beyond 2 or 3 mm in diameter.<sup>40</sup> Folkman proposed that the acquisition of an "angiogenic switch" was necessary for tumor growth and metastasis.<sup>41</sup> He first recognized this as an important factor in the development of other chronic diseases, such as atherosclerosis, chronic liver disease, and rheumatoid arthritis. This has led to the development of a huge range of antiangiogenic therapies, some of which are now in widespread clinical use, including small molecules and monoclonal antibody inhibitors.<sup>42,43</sup> Viruses have been recognized as offering the possibility of targeted delivery of angiogenesis inhibitors, providing local expression of these proteins on a continual basis in order to maximize efficacy and limit side effects. This review will focus on the angiogenesis inhibitors delivered by replicating OV<sub>s</sub> for cancer therapy, forming an update from our previous review on this topic.<sup>44</sup>

## Specific importance of angiogenesis in cancer

Angiogenesis is regulated by the balance of angiogenic growth factors and inhibitors, which are released from endothelial cells (ECs), monocytes, platelets, and smooth muscle and tumor cells.<sup>45</sup> The normal physiological situation in solid organ vasculature is an excess of inhibitors. When an excess of growth factors is present, as is frequently the case in tumors, the balance is tipped in favor of angiogenesis.

Vascular endothelial growth factor (VEGF) and other growth factors released from tumors bind to receptors on both the endothelial cells of nearby blood vessels and circulating bone marrow-derived epithelial progenitor cells, resulting in their activation, proliferation, and production of enzymes.<sup>46</sup> The resulting enzymes dissolve holes in the basement membrane of the surrounding blood vessels, allowing proliferating ECs to migrate out toward the tumor. MMPs produced by ECs dissolve the surrounding stroma, allowing the ECs to advance toward the tumor, using integrins including  $\alpha v\beta 3$  and  $\alpha v\beta 6$  to direct them.<sup>47</sup> The ECs then remodel and form tubes, which connect into loops through the tumor mass, so forming complete blood vessels. Structural support cells, such as smooth muscle cells, then follow, although tumor blood vessels remain leaky and have a poorly formed basement membrane, two factors that have been proposed to aid the inherent tumor specificity of some viral vectors.

The development of angiogenesis inhibitors has become a broad and active area of cancer research.<sup>45,48–51</sup> A wide range of angiogenesis inhibitors have been discovered and the most widely studied include protease inhibitors, tyrosine kinase inhibitors, chemokines, interleukins (ILs) (eg, IL-8, IL-12, IL-18), and proteolytic fragments of diverse molecules (eg, endostatin, angiostatin, vacuostatin, canstatin). These antiangiogenic molecules function in multiple ways, including inhibiting endothelial cell proliferation, migration, protease activity and tubule formation, as well as inducing apoptosis.

Antiangiogenic agents offer lower toxicity than most conventional chemotherapy, allowing long-term use.<sup>48,52</sup> VEGF is the most commonly targeted angiogenic growth factor, and the first angiogenesis inhibitor to be licensed in Europe was bevacizumab. This class I inhibitor is an antibody that specifically blocks VEGF and was licensed in January 2005 for the treatment of metastatic colorectal carcinoma, following its success in clinical trials.<sup>53</sup> However, resistance has been seen in patients with other tumor types, where multiple angiogenic factors may be produced by primary tumors.<sup>54</sup>

Despite great promise, the results obtained with the use of these peptide inhibitors alone in clinical trials have been disappointing, and regimes combining angiogenesis inhibitors with standard chemotherapeutic regimes are often required.<sup>42,43</sup> Many angiogenesis inhibitors are not directly cytotoxic to tumor cells, but need to be expressed on a continuous basis to inhibit ECs effectively. Initial efforts focused on the targeting of VEGF- and tumor-derived VEGF-signaling. However, resistance was observed, and a greater understanding of the mechanisms of drug resistance<sup>50</sup> and the concept

that sustained local delivery of angiogenesis inhibitors to tumors may be more effective has led to the development of antiangiogenic cancer gene therapy.<sup>45</sup>

## Targeting endothelial cells with oncolytic viruses

Some OV's display an innate ability to infect ECs in tumors, while sparing those in normal vessels.<sup>55</sup> Intravenous delivery of a vesicular stomatitis virus in a murine colorectal carcinoma xenograft model showed direct infection of ECs. This induced neutrophil infiltration, leading to microclot formation with tumor-associated vasculature, resulting in a large bystander effect of cell death within the tumor.<sup>56</sup> An HSV delivered intravenously in a murine ovarian carcinoma model has also been found to specifically infect tumor-associated ECs, causing cell death while sparing the ECs in normal organs.<sup>57</sup> In a Phase I clinical trial, intravenous delivery of oncolytic VV engineered to target cells with activation of the Ras/mitogen-activated protein kinase (MAPK) pathway was found to selectively infect tumor-associated ECs, sparing normal ECs.<sup>58</sup> This provides a useful platform for the further development of OV's armed with therapeutic transgenes in the future.

## Arming oncolytic viruses with angiogenesis inhibitors

While nanoparticles, liposomes, and naked plasmid DNA electroporation have all been used to deliver antiangiogenesis gene therapy, it is viruses that are the most promising vectors for the delivery of angiogenesis inhibitors.<sup>59</sup> The ability of OV's to infect and selectively amplify the input dose of virus in the target tumor has been exploited to address some of the limitations of nonreplicating viruses.<sup>23,60</sup> OV's can selectively target and kill tumor ECs as well as tumor cells, although there is evidence that they can themselves increase vascular permeability in tumors, stimulating angiogenesis.<sup>20</sup> The combination of antiangiogenic therapy delivered by an OV may prevent this through reduction of the host immune response, by stabilizing tumor vasculature and decreasing immune cell infiltration.<sup>21</sup> The selective expression of angiogenesis inhibitors in the tumor microenvironment prevents further tumor growth, allowing the viral progeny produced through replication to spread through tumors, infecting and inducing lysis of cancer cells in order to achieve tumor clearance.

Since our last review on this subject was published,<sup>44</sup> there have been 17 new OV's armed with antiangiogenic genes reported in animal models, making a total of 32 studies in



the literature (Tables 1–3). There are still no clinical trials reported nor registered to date.

## Targeting VEGF

VEGF has a key role in the signaling pathways that mediate angiogenesis, tumor growth, and metastasis. Monoclonal antibodies against VEGF are now in widespread clinical use in oncology. Since VEGF is highly expressed in many cancers, this pathway has been targeted by many OV's expressing angiogenesis inhibitors. The first reported OV expressing an angiogenesis inhibitor was a first-generation oncolytic adenovirus (ONYX-015, E1B55k, and E3B-deleted adenovirus) armed with a soluble VEGF-receptor inhibitor, sFlt-1, which was effective in an animal model of human colorectal cancer.<sup>62</sup> The combination of a second-generation oncolytic adenovirus (dl922/47, with *E1ACR2* mutation and *E3B* deletion) and a nonreplicating adenovirus expressing Flk1-Fc, a soluble ectodomain of the VEGF receptor, was more effective than either virus alone.<sup>63</sup> Another E1B55 kDa-deleted oncolytic adenovirus was effective in a human colorectal model by expressing vascular endothelial growth inhibitor (VEGI).<sup>64</sup> Ad-Δ7B-KOX (E1B19 kDa and E1B55 kDa gene-deleted), an oncolytic adenovirus expressing a VEGF promoter targeted artificial zinc-finger protein inserted into the *E3* gene, reduced VEGF expression, and increased the survival of animals bearing human glioblastoma xenografts.<sup>65</sup>

More recently, a group has published results of both oncolytic adenovirus<sup>66</sup> (Ad5/3 serotype with hypoxia inducible factor [HIF]-promoter) and VV<sup>67</sup> (TK- and vaccinia growth factor-deleted) expressing VEGF-1-immunoglobulin (Ig), a soluble inhibitor that binds VEGF without inducing vascular EC mitogenesis.<sup>68</sup> VV has also been used to deliver a single chain antibody to VEGF, which was effective in a canine

xenograft model.<sup>69</sup> Targeting the VEGF pathway has been effective in animal models and shows promise for translation to clinical studies in the future.

## Targeting interleukins and chemokines

IL-24 is a good candidate for expression by OV's, as it is an effective antiangiogenic cytokine and as well, induces apoptosis and reduced growth in many tumors.<sup>70,71</sup> Three different oncolytic adenoviruses have been engineered to express IL-24,<sup>72–74</sup> with coexpression of arrestin, and these were effective in a melanoma model.<sup>74</sup> IL-8 has been targeted, as it promotes angiogenesis, tumor growth, and metastasis.<sup>75</sup> An oncolytic adenovirus delivering small interfering ribonucleic acid (siRNA) against IL-8 was effective in a range of human xenograft models, including metastatic breast cancer.<sup>76</sup> IL-18 has also been identified as an angiogenic inhibitor and tumor suppressor.<sup>77</sup> An oncolytic E1b55 kDa-deleted adenovirus expressing IL-18 was effective in a human renal carcinoma xenograft model. IL-12<sup>78</sup> and chemokine platelet factor 4 (PF4)<sup>79</sup> are also potent antiangiogenic agents, and oncolytic HSVs armed with IL-12 or PF4 have shown promise in animal models.<sup>80–82</sup>

## Targeting matrix metalloproteinases

MMPs play a pivotal role in angiogenesis by degrading the stroma of the ECM that surrounds blood vessels, leading to EC proliferation, migration, and new capillary formation.<sup>83</sup> Tissue inhibitors of metalloproteinases (TIMP) have been developed for antiangiogenic therapy. The only reported virus expressing a TIMP is AdΔ24TIMP-3, an oncolytic adenovirus expressing TIMP-3. However, despite reducing levels of MMP-2, this did not lead to decreased tumor growth or improved survival in a human glioma model.<sup>84</sup>

**Table 1** Oncolytic viruses expressing inhibitors of the VEGF pathway

Oncolytic virus and angiogenesis inhibitor expressed	Tumor model	Reference
ZD55-VEGI-251, oncolytic adenovirus (E1B55 KDa deleted) expressing VEGI	Human cervical and colorectal xenografts	Xiao <sup>64</sup>
vvdd-VEGFR-I-Ig, oncolytic vaccinia virus (thymidine kinase and vaccinia virus growth factor deleted) expressing VEGFR-I-Ig fusion protein	Murine renal cancer	Guse <sup>67</sup>
Ad5/3-9HIF-Delta24-VEGFR-I-Ig, oncolytic adenovirus (5/3-serotype chimera) expressing VEGFR-I-Ig fusion protein	Murine renal cancer	Guse <sup>66</sup>
Oncolytic adenovirus dl922/47 (E1A CR2 region mutated) + nonreplicative adenoviral vector expressing Flk1-Fc	Human colon and prostate cancer	Thorne <sup>63</sup>
Ad-ΔB7-KOX, oncolytic adenovirus (E1B19 kDa and E1B55 kDa gene-deleted) expressing VEGF promoter-targeted transcriptional repressor ZFP	Human glioblastoma	Kang <sup>65</sup>
ZDD-sflt-1, oncolytic adenovirus (E1B55 KDa deleted) expressing sflt-1 (the first three extracellular domains of FLT1, the VEGF receptor-1)	Human colon cancer	Zhang <sup>62</sup>
GLV-1h109, oncolytic vaccinia virus expressing a single-chain antibody to VEGF	Canine sarcoma and prostate cancer	Patil <sup>69</sup>

**Abbreviations:** Ig, immunoglobulin; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; VEGI, vascular endothelial growth inhibitor; vv, vaccinia virus; ZFP, zinc finger protein.

**Table 2** Oncolytic viruses expressing cytokines and chemokines

Cytokines and chemokines	Oncolytic viruses and genetic modification	Tumor model	References
Interleukin-18	ZD55-IL-18, oncolytic adenovirus (E1B55 KDa deleted) expressing IL-18	Human renal cancer xenografts	Zheng <sup>106</sup>
Interleukin-24	Ad.DD3-E1A-IL-24, oncolytic adenovirus (DD3 replaces E1A promoter) expressing IL-24	Human prostate cancer	Fan <sup>101</sup>
Interleukin-24	Ad-sp-E1A((Δ24))-E1B((Δ55))-IL-24, oncolytic adenovirus (E1A 24 kDa and E1B 55 kDa deleted) expressing IL-24 under the surviving promoter	Human lung xenografts	Xiao <sup>73</sup>
Interleukin-24	Conditionally replicating adenovirus expressing IL-24 and arrestin	Human melanoma	Chai <sup>74</sup>
Interleukin-8	Ad-ΔB7-U6shIL8, oncolytic adenovirus (E1B55 KDa deleted) expressing IL-8-specific shRNA	Human solid tumors	Yoo <sup>76</sup>
Interleukin-12	NV1042, oncolytic HSV (NV1023) expressing murine IL-12	Murine prostate, head and neck cancer	Varghese, <sup>81</sup> Wong <sup>82</sup>
Platelet factor 4	bG47Delta-PF4, oncolytic HSV expressing PF4	Human and mouse glioma	Liu <sup>104</sup>
Tissue inhibitor of metalloproteinase-3	AdΔ24TIM-3, oncolytic adenovirus (E1A CR2 region-mutated, aka, Δ24 or dI922/47) expressing TIMP3 gene	Human glioma	Lamfers <sup>84</sup>

**Abbreviations:** HSV, herpes simplex virus; IL, interleukin; PF4, platelet factor 4; shRNA, short hairpin ribonucleic acid; TIMP3, tissue inhibitor of metalloproteinase-3.

**Table 3** Oncolytic viruses expressing other endogenous inhibitors

Endogenous inhibitor	Oncolytic viruses and genetic modification	Tumor model	Reference
Endostatin/angiostatin	VVhEA, oncolytic Lister strain vaccinia virus expressing human endostatin-angiostatin fusion protein	Human head and neck cancer	Tysome <sup>99</sup>
	Ad-hE, oncolytic adenovirus expressing human endostatin encapsulated in PEG-PE cationic liposome	Human ovarian cancer	Yang <sup>93</sup>
	G47Δ-mAngio, oncolytic herpes simplex virus expressing murine angiostatin in combination with bevacizumab	Human glioma	Zhang <sup>94</sup>
	rAAV-TIE, oncolytic adeno-associated virus expressing endostatin and HSV-thymidine kinase	Human bladder cancer	Pan <sup>91</sup>
	VVhEA, oncolytic Lister strain vaccinia virus arming with human endostatin-angiostatin fusion protein gene	Human pancreatic cancer	Tysome <sup>98</sup>
	EndoAngio-PRRA, a prostate-restricted, replication-competent adenovirus expressing endostatin-angiostatin fusion gene	Human prostate cancer	Li <sup>96</sup>
	HSV-Endo, oncolytic HSV-I mutant expressing murine endostatin gene	Human colon cancer	Mullen <sup>88</sup>
	AE618, an oncolytic HSV-I G027 expressing endostatin-angiostatin fusion protein	Human lung cancer	Yang <sup>23</sup>
	CNHK200-mE, oncolytic adenovirus (E1B55 KDa deleted) expressing mouse endostatin	Human hepatocellular carcinoma	Li <sup>86</sup>
	CNHK300-mE, oncolytic adenovirus (hTERT-promoter to drive the expression of the adenovirus E1A gene) expressing mouse endostatin	Human hepatocellular carcinoma and gastric cancer	Li, <sup>87</sup> Zhang <sup>90</sup>
	CNHK500-mE, oncolytic adenovirus (E1a and E1b genes controlled by the human telomerase reverse transcriptase promoter and the hypoxia response element) expressing mouse endostatin	Human nasopharyngeal cancer	Su <sup>89</sup>
	HSV-Endo, oncolytic herpes simplex virus-I expressing endostatin	Murine lung cancer	Goodwin <sup>92</sup>
	RAMBO expressing vaculostatin (Vstat120) under control of the HSV IE4/5 promoter	Human glioma	Hardcastle <sup>103</sup>
Plasminogen kringle 5	ZD55-mK5, oncolytic adenovirus (E1B55 KDa deleted) expressing mutated kringle 5 of human plasminogen)	Human colon cancer	Fan <sup>101</sup>
Canstatin	Oncolytic adenovirus (E1B55 KDa deleted) expressing canstatin	Human pancreatic cancer	He <sup>102</sup>
Fibroblast growth factor receptor	bG47Delta-dnFGFR, oncolytic herpes simplex virus arming with a dominant-negative FGF receptor	Human glioma and mouse malignant peripheral nerve sheath tumor	Liu <sup>104</sup>

**Abbreviations:** FGF, fibroblast growth factor; hTERT, human telomerase reverse transcriptase; HSV, herpes simplex virus; PEG-PE, phospholipid derivative of polyethylene glycol; RAMBO, rapid antiangiogenesis mediated by oncolytic virus.

## Other endogenous inhibitors

Endostatin has displayed the broadest anticancer spectrum of all endogenous inhibitors currently identified and inhibits migration of tumor cells and ECs as well as invasion of tumor cells.<sup>85</sup> The endostatin gene has been engineered to be expressed by oncolytic adenovirus, adeno-associated virus and HSV,<sup>86–93</sup> showing better efficacy than control or nonreplicating viruses expressing endostatin.

Angiostatin, a 38 kDa fragment of plasminogen, inhibits EC proliferation and migration as well as inducing apoptosis. The strategy of combining angiogenesis inhibitors that work through different pathways has been investigated since they tend to exhibit a low side-effect profile when compared with conventional chemotherapy. An oncolytic adenovirus expressing angiostatin has shown promise when delivered locally following systemic bevacizumab.<sup>94</sup> Endostatin and angiostatin have been found to act synergistically when used in combination, which led to the development of an endostatin-angiostatin fusion gene.<sup>95</sup> This fusion gene has been incorporated into oncolytic HSV, adenovirus, and VV.<sup>96–98</sup> We found that oncolytic VV expressing the fusion protein displayed superior efficacy over ONYX-015 in a head and neck cancer model.<sup>99</sup>

Another fragment of plasminogen, kringle 5, inhibits EC proliferation more effectively than angiostatin.<sup>100</sup> Kringle 5 and a mutant kringle 5 (mK5, with leucine71 changed to arginine) have been delivered by a first-generation oncolytic adenovirus (ONYX-015, E1B55 kDa-deleted).<sup>101</sup> The ZD55-mK5 virus significantly suppressed tumor growth and improved survival in a human colorectal xenograft model. ONYX-015 has also been used to express canstatin, a 24 kDa fragment of type IV collagen.<sup>102</sup> An oncolytic HSV expressing vasculostatin, a fragment of brain-specific angiogenesis inhibitor-1, has shown promise in a human pancreatic model.<sup>103</sup>

Fibroblast growth factor (FGF) signaling is another important mediator of both EC and tumor cell migration essential in angiogenesis. A novel oncolytic HSV armed with a dominant-negative FGF receptor has been developed and has been shown to be more effective than its unarmed counterpart at inhibiting tumor growth and angiogenesis, in both human and mouse tumor models *in vivo*.<sup>104</sup>

## Future developments

Many different angiogenesis inhibitors have been used to arm OV. Most have shown promise in animal models, but none have reached clinical trials. The major barriers limiting the

efficacy of OV are still tumor selectivity, the effect on the tumor microenvironment, and the host immune response to virus-infected tumor cells.<sup>9</sup> Many strategies are being developed to overcome these obstacles and to optimize OV delivery. Lessons should be learnt from standard chemotherapeutic regimes, which often combine multiple agents with different mechanisms of action. The same approach should be used with angiogenesis inhibitors, where less toxicity is usually observed when compared with most standard chemotherapy drugs.

Many antiangiogenic drugs have the ability to constrict abnormal leaky tumor vessels.<sup>105</sup> This ability to temporarily normalize tumor vasculature provides a therapeutic window during which systemic delivery of OV may be improved. Clinical studies should concentrate on combining OV with standard treatment regimes in order to enhance their efficacy.

## Disclosure

The authors report no conflicts of interest in this work.

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