Expert Opinion

Vector therapies for malignant glioma: shifting the clinical paradigm

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Background: Malignant glioma represents one of the most aggressive and devastating forms of human cancer. At present, there exists no successful treatment for this disease. Gene therapy, or vector therapy, has emerged as a viable experimental treatment method for intracranial malignancies. *Objective*: Vector therapy paradigms that have entered the clinical arena have shown adequate safety; however, the majority of the studies failed to observe significant clinical benefits. As such, researchers have refocused their efforts on developing novel vectors as well as new delivery methods to enhance the therapeutic effect of a particular vector. In this review, we discuss common vector therapy approaches used in clinical trials, their drawbacks and potential ways of overcoming these challenges. *Methods*: We focus on the experimental evaluation of cell-based vector therapies and adenoviral and herpes simplex virus type 1 vectors in the treatment of malignant glioma. *Conclusion*: Vector therapy remains a promising treatment strategy for malignant glioma. Although significant questions remain to be answered, early clinical data suggest safety of this approach and future studies will likely address the efficacy of the proposed therapy.

Keywords: adenovirus, brain tumor, cancer, clinical trial, gene therapy, glioma, herpes simplex virus type 1, MRI, PET, vector therapy

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1. Introduction

Malignant glioma represents the most common type of primary brain cancer [1]. These tumors commonly demonstrate molecular atypias associated with the amplification of growth factor cascades [2], p53 dysregulation [3-5], pRb malfunction [6-8] and INK4a/ARF alterations [8-11], among others. These molecular abnormalities accumulate during malignant progression and give rise to endothelial hyperplasia, high indices of mitosis, infiltration and necrosis upon pathological investigation.

Despite aggressive treatment regimens including gross total resection, radiation therapy and concomitant temozolomide, the prognosis of patients diagnosed with malignant glioma remains bleak, with an average time of survival approximating 14 months [12]. Recent discoveries in molecular biology have prompted researchers and physicians to seek out novel approaches for the treatment of malignant glioma. One such approach that has undergone much investigation is gene therapy, or vector therapy. The physioanatomical make up of the CNS renders primary malignant gliomas a model disease for the evaluation of vector therapy. First, the CNS constitutes an area of 'immune privilege', making delivery of allogenic agents feasible. Second, primary gliomas rarely metastasize outside of the CNS, allowing for local delivery of experimental vector therapies.

Recent clinical trials employing cell-mediated delivery and viral delivery of vectors established the safety and practicality of vector therapy for malignant glioma;

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however, investigators failed to observe significant anti-tumor effects resulting from these approaches. Nonetheless, vector therapy remains a provocative treatment modality for malignant glioma and researchers have focused their efforts on addressing the setbacks encountered in clinical trials. In this review, we discuss some commonly cited obstacles encountered in clinical trials and then take a look at representative studies which suggest promising solutions to these problems. Specifically, we will focus on some of the more commonly studied vector delivery paradigms for malignant glioma, those being cell-mediated delivery, adenoviral and herpes simplex type-1 (HSV-1) vectors, with more attention being paid to oncolytic adenoviral and HSV-1 vectors, as they have become the more predominately studied vector therapies for malignant glioma.

2. Cell-mediated gene delivery

Several clinical trials employing cell-mediated delivery of vectors to malignant glioma employed vector packaging cells (VPCs) that were engineered to deliver a therapeutic gene in the form of a replication-deficient Maloney murine leukemia retrovirus vector (MLV) (**Figure 1**) [13]. MLV only integrates into cells with disintegrated nuclear membranes (i.e., dividing cells) and can carry a gene of interest of up to 8 kb, making it an attractive vector for tumor cell-specific transduction and gene expression. MLV vectors are made replication-deficient by deleting the essential *gag* (capsid proteins), *pol* (reverse transcriptase) and *env* (envelope proteins) from the MLV genome, leaving the long terminal repeats (LTRs), packaging sequence (ψ), primer binding site (PBS; for initiation of reverse transcription) and gene of interest alone. Vector packaging cells are named as such because they have been engineered with the essential retroviral genes *gag*, *pol* and *env*. Upon transfection of VPCs with a replication-deficient MLV vector, complete, packaged MLV virions carrying the gene of interest are produced. Once VPCs are injected into an area within the vicinity of the tumor, MLV virions bud from the VPC membrane, transduce neighboring malignant cells and deliver their therapeutic payload by random insertion into the host genome.

The earliest and most common gene therapy trials for malignant glioma involved the application of prodrug, or 'suicide' gene delivery by VPCs (**Figure 1**) [14]. Frequently practised prodrug gene therapy consists of VPC-mediated delivery of MLV virions carrying the herpes simplex virus-1 gene, thymidine kinase (*tk*), to intracranial cavities after gross total resection followed by intravenous (i.v.) administration of the nucleoside analogue, ganciclovir (GCV) [15]. Given its delivery vehicle (MLV virion), the viral TK – a nucleotide synthesis enzyme – should be selectively active in cells with an overactive rate of division, such as glioma cells [16]. After administration of GCV into herpes simplex virus-thymidine kinase (HSV-tk)-transduced cells, GCV is phosphorylated by viral and cellular thymidine kinases and competitively inhibits the activity of DNA synthesis enzymes, thereby killing tumor cells in a selective manner (**Figure 1**) [17,18]. An important facet of prodrug therapy's functionality centers on what is referred to as the bystander effect, whereby neighboring non-transduced tumor cells are subject to the toxicity of GCV (**Figure 1**) [17,18].

While clinical trials involving cell-based methods demonstrated adequate safety, post-engraftment biopsies and autopsies have revealed poor transduction levels, ranging from below 0.002% to a maximum of 2.6% in some studies [19,20]. Investigators propose that this low transduction rate was partly the result of the vector producing cell and the vector itself [19].

The VPCs chosen for malignant glioma clinical trials were generally of murine fibroblast origin, a phenotypically non-motile cell type [20,21]. It was suggested that the non-motile characteristic of a VPC hinders the ability of vectors to reach diffuse tumorigenic regions and reduces transgene expression [20,22]. In light of this, researchers have investigated the potential utility of 'gliomatropic' stem cells [23,24]. Given the characteristic migratory capacity of stem cells and the diffuse nature of malignant glioma, stem cells logically surfaced as a viable option for delivering transgenes to diffuse tumor microsatellites. However, an important distinction to make is that recent research involving the use of stem cell-mediated delivery of therapeutic genes does not involve engineering cells to package and deliver a gene in the form of retroviral virion and thus they are not considered VPCs per se. This may be because free retroviral vectors are destroyed by complement activation, making their use in glioma therapy applications less than practicable (**Figure 1**) [25]. Studies mentioned below involve human stem/progenitor cells that have been manipulated to express only the therapeutic gene by itself and not in the form of a replication-deficient retroviral virion.

Neural stem cells (NSCs) are of interest for vector delivery to malignant glioma, given their innate migratory ability and tropism for intracranial pathologies (**Figure 2**) [26-30]. Brown *et al.* illustrated the migratory aptitude and responsiveness of NSCs to glioma pathology when she demonstrated that murine NSCs injected into the tail vein of nude mice could engraft into both intracranial tumors *and* flank-established tumors in the same animal [31]. NSCs have also demonstrated the capability to effect tumor regression by targeting prodrug therapies, 'immuno-genes' and replication-deficient adenoviruses (Ads) bearing transgenes to malignant glioma [32-35]. In a recent study, Li *et al.* demonstrated the ability of NSCs to mediate a bystander effect via transfer of HSV-TK, resulting in substantial tumor regression and 67% long-term survival (n = 6/9) in Sprague–Dawley rats bearing intracranial gliomas [36].

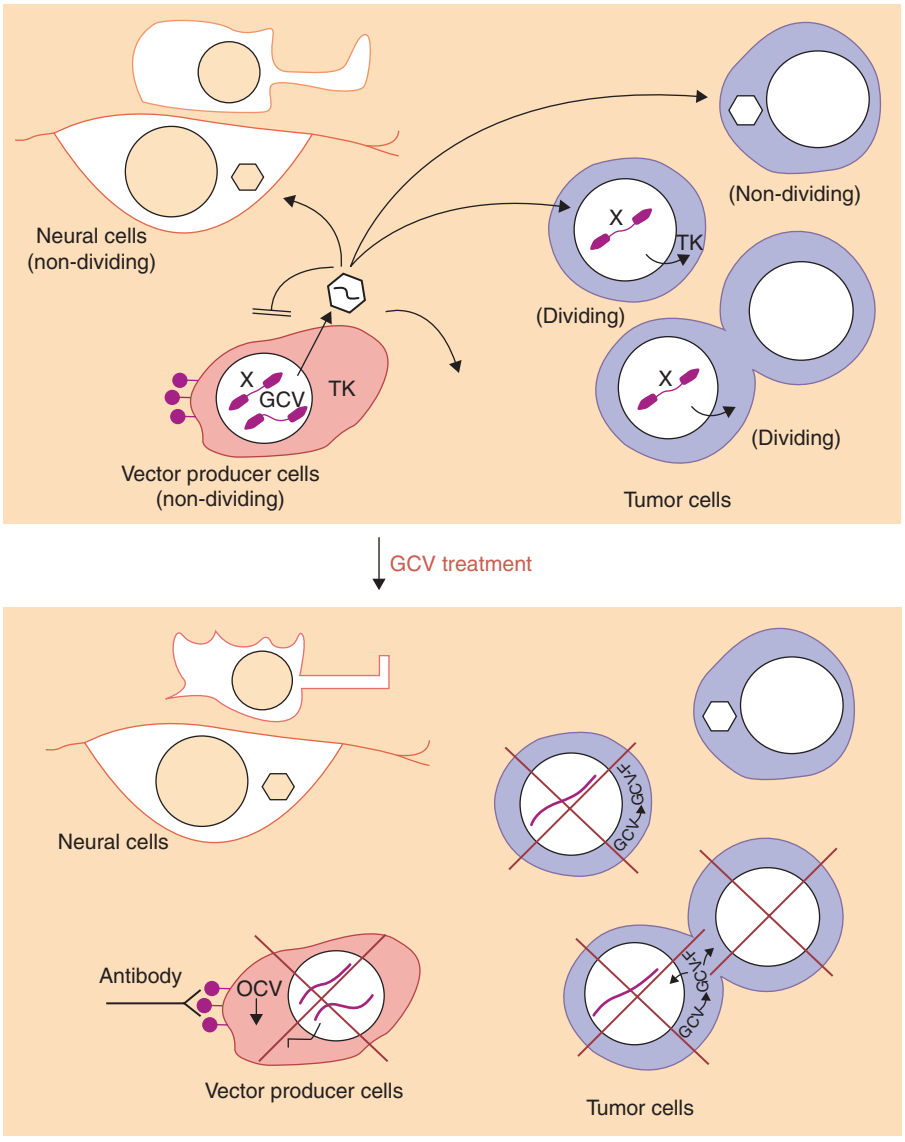

Figure 1. The prodrug paradigm. Vector producing cells (VPCs) are delivered into the tumor cavity after surgical resection. VPCs are engineered to express the *gag*, *pol*, *env* genes from their genome and complement the packaging of replication-deficient MLV virions carrying the HSV-*tk* gene. By nature, MLV vectors only integrate into dividing cells, making their activity selective for rapidly dividing tumor cells. MLV will transduce and then integrate their truncated genome, which includes the prodrug gene HSV-*tk*, into the tumor cell genome. Ideally, the tumor cell should then express the HSV-*tk* gene and, upon GCV administration, will 'commit suicide' (top). The host immune system recognizes and destroys the murine VPCs and virions; however a key feature of the prodrug paradigm is the bystander effect, in which non-transduced cells are susceptible to GCV toxicity (bottom slide).
Figure kindly provided by [120].
GCV: Ganciclovir.

Mesenchymal stem cells (MSCs) and bone marrow-derived stem cells (BMDSCs) also represent a feasible paradigm for enhanced gene delivery to malignant glioma. MSCs and BMDSCs migrate and engraft into areas of tumorgenesis and contribute to enhanced neovascularization in response to malignant glioma pathophysiology [37]. Malignant glioma can also recruit MSCs by secreting factors such as IL-8, TGF-β1, neurotrophin-3, VEGF, PDGF-BB and EGF [38-40].

MSCs and BMDSCs also possess the capacity to deliver therapeutic genes to malignant glioma [41]. In a representative study, Miletic *et al.* showed the capability of bone marrow-derived progenitor cells (BMPCs) to infiltrate intracranial malignancies in rats and deliver the HSV-*tk* gene. In this study, mice receiving BMDPCs expressing TK and receiving administration of GCV had a 60% long-term survival rate [42]. And while studies involving the use of VPCs for glioma therapy have abated

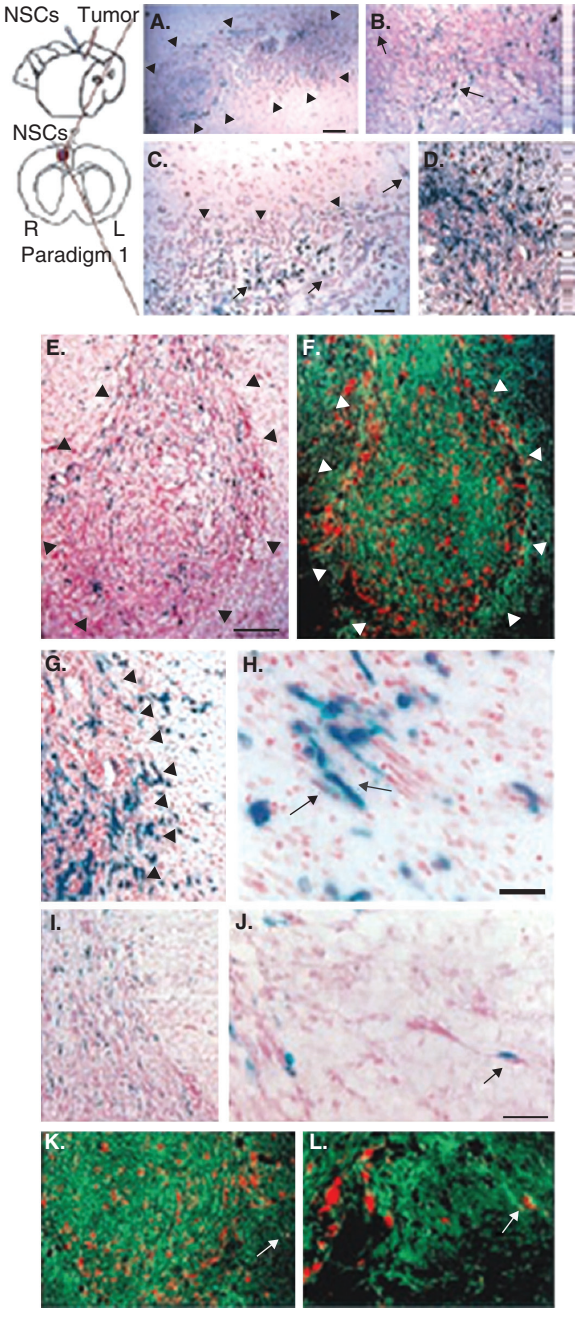

Figure 2. Stem cells migrate and engraft into intracranial glioma. Stem cells are attractive agents for targeted vector delivery because they respond to the pathophysiology of malignant glioma. This figure demonstrates that human neural stem cells (NSCs; denoted by red and blue markers with immunofluorescent and immunohistochemical stainings, respectively) are capable of extensive engraftment throughout the tumor region.
Figure reprinted with permission from [121].

as of late, a recent study by Fischer *et al.* demonstrated that bone marrow tumor infiltrating progenitor cells (BM-TICs) isolated from rats could be engineered into cells capable of producing lentiviral vectors. These cells demonstrated appreciable glioma engraftment as well as specific and stable transduction of glioma [43].

3. Viral vector delivery

At present, studies involving the use of HSV-1 and Ad vectors constitute a significant majority of research in the field of vector therapies for malignant glioma. The remainder of this review will provide a brief overview of what has been evaluated in malignant glioma clinical trials followed by a look at recent developments with these two vectors.

3.1 Biology of adenovirus and HSV-1

3.1.1 Adenovirus

The human adenovirus type 5 (Ad5) virion consists of a naked (wholly protein) icosahedral capsid 70 – 90 nm in diameter, with knobbed fibers extending from each of the 12 vertices of the capsid. A total of 11 different proteins comprise the outer capsid structure and inner nucleocapsid which is complexed with a linear double stranded genome 30 – 36 kb in length. The knob region of the Ad fiber mediates primary binding to its native receptor, the coxsackie and Ad receptor (CAR); secondary receptor binding and subsequent internalization is mediated by $\alpha_v\beta_3$ integrin binding to RGD (Arg-Gly-Asp) motifs located at the base of the fiber region. Upon receptor-mediated endocytosis, Ad5 undergoes endosomal transport to nuclear pores where its genome is released into the nucleus. Ad5 gene expression is ordered in a sequential fashion: first, the early genes are expressed (E1A, E1B, E2A, E2B, E3, E4) along with intermediate gene expression (IX, Iva2), followed by late gene expression (L1-L5). RNA splicing events of Ad5 transcripts produces different mRNA species which give rise to approximately 50 different viral proteins. The early and intermediate Ad5 genes coordinate the coercion of host cells to generate an optimal environment that allows for viral genome amplification and progeny production. Shortly after Ad5 genome replication, viral proteins mediate the transcription of the late genes, which encode structural constituents of the virion capsid and inner core.

Ad5 vectors are feasible for therapeutic application given their high-titre production, large transgene capacity, adeptness for transducing multiple cell types – both dividing and non-dividing – high rate of transgene expression and the fact that their genome remains episomal, obviating the possibility of host mutagenesis that might occur with other vector delivery systems [44].

3.1.2 HSV-1

HSV-1 life cycle has been extensively reviewed [45,46]. Briefly, the HSV-1 virion consists of an enveloped (lipid membrane) icasohedral capsid 125 nm in diameter. Residing between the envelope and protein capsid is a mixture of proteins named the tegument; little is known regarding the discrete function of tegument proteins. The capsid is comprised of six different

proteins which house a linear double-stranded DNA genome 120 – 250 kb in length. The envelope contains approximately 10 different glycoprotein constituents. Viral glycoproteins gB and gC initiate cell surface attachment through host heparan sulfate proteoglycans, after which gD binds to receptors such as nectin 1 and herpes virus entry mediator (HVEM) [47-49]. Upon cell surface binding, glycoproteins gB, gI and gH mediate fusion-dependent viral entry after which microtubule-mediated translocation to the nucleus and subsequent genome delivery occurs [50,51]. HSV-1 genes are categorized into three groups based on the sequential order of their expression: first, the immediate early genes (α genes) are expressed, followed by the β genes, which are important for viral genome amplification, and finally, the late genes, or γ genes, are expressed, which make up the majority of the HSV-1 genome.

HSV-1 is an attractive vector for malignant glioma because it is naturally neurotropic [52,53]. Proponents of HSV-1 oncolytic therapy believe this characteristic makes it a suitable vector for primary CNS tumors. In addition, HSV-1 genome is relatively large compared to other commonly used vectors, and multiple portions of its genome can harbor modifications without substantially affecting viral titre [53].

Both replication-deficient and replication-competent Ad5 vectors have been tested in clinical settings, while only replication-competent HSV-1 vectors have been clinically appraised for malignant glioma [54-59]. Deficient Ad5 vectors have been engineered to deliver genes such as HSV-*tk* and *p53* to malignant glioma in the clinical setting; however, low transgene expression and short-term therapeutic activity have discouraged researchers from further evaluation of these vectors for clinical applications [14]. As such, we omit discussion of these vectors here; instead, our discussion will apply to competent viral vectors.

3.2 Oncolytic viral vectors

Replication-competent viral vectors often do not harbor therapeutic transgenes such as *p53* or HSV-*tk*. Rather, competent vectors exploit the viral lytic cycle to achieve sustained glioma cell lysis, a process termed oncolysis, or virolysis. Competent vectors are engineered to specifically transduce, replicate and spread in tumorigenic regions such that a logarithmic killing effect of glioma cells is obtained, ceasing only at the point of complete tumor eradication. To date, there exist three prevailing strategies that have been employed to achieve glioma-targeted viral replication and oncolysis, or "conditional replication" and they are: i) those involving cancer complement vectors, which have been tested in clinical trials, and more recently, those involving vectors with; ii) transcriptional; and iii) transductional (viral entry) modifications.

3.2.1 *First generation oncolytic vectors: cancer complement vectors*

Cancer complement vectors are a class of competent viral vectors whose replication is made tumor-specific via deletion of viral genes, or regions of a viral gene, that are necessary for non-specific and potentially dangerous replication (i.e., in non-dividing cells such as neurons), but dispensable for replication in rapidly dividing tumor cells. The high mitotic index of malignant glioma renders many glioma cells in a pro-growth status, a favorable environment for viral replication. The increased availability of DNA metabolism enzymes and proteins in malignant cells 'complements' viral mutational deletions *in trans*, enabling viral replication and cytotoxicity to occur in a specific fashion [14]. Non-neoplastic cells – those with low mitotic indices, such as neurons – remain non-permissive to cancer complement vectors. Commonly cited replication-competent vectors falling under this category include ONYX-015 (Ad), G207 (HSV-1) and 1716 (HSV-1) [54,57,60].

3.2.2 *Cancer complement vectors in the clinical setting: safe but weak*

The conditionally replicative Ad5 vector ONYX-015 was made tumor-specific by deletion of the 55K E1B protein, which is responsible for binding p53 early in the Ad5 life cycle and inhibiting p53-mediated transcriptional regulation and consequent cell death [61]. The replication of ONYX-015 was hypothesized to be selective for glioma cells with deleterious p53 pathways; however, recent studies suggest alternate mechanisms [62]. Preclinical data demonstrating the promise of ONYX-015 were sufficient enough for it to enter into a Phase I clinical trial [54].

Similarly, virulent proteins responsible for non-specific viral cytotoxicity have been removed from competent HSV-1 vectors to render them safe and glioma-specific. These include both copies of the $\gamma_1 34.5$ gene and the $U_L 39$ gene. $\gamma_1$34.5 is essential for robust HSV-1 progeny production (oncolysis) and acts by binding protein phosphatase 1α, preventing downstream activation of PKR, NF-κB and eventual antiviral immunogenicity [63,64]. U_L39 (ICP6) encodes the large subunit of a viral ribonucleotide reductase, which is important in nucleotide metabolism [65]. Vectors carrying these deletions selectively replicate in glioma cells with aberrant protein synthesis and elevated nucleotide pools [63-68]. The clinically evaluated G207 vector carries deletions in both $\gamma_1$34.5 loci along with a lacZ insertion in the U_L39 coding region [57].

While the safety of the above-mentioned competent vectors has been verified, the lack of reporting on the biological efficacy of tested applications represents an inherent limitation of Phase I/II endeavors. Nonetheless, these studies failed to show a significant anti-tumor response resulting from vector delivery [54,57]. Researchers suggest the failure to observe significant benefits from these vectors could possibly be from poor vector replication and spread resulting from the attenuation of viral genomes, inadequate tumor transduction, host immune reaction and insufficient protocols [25,69].

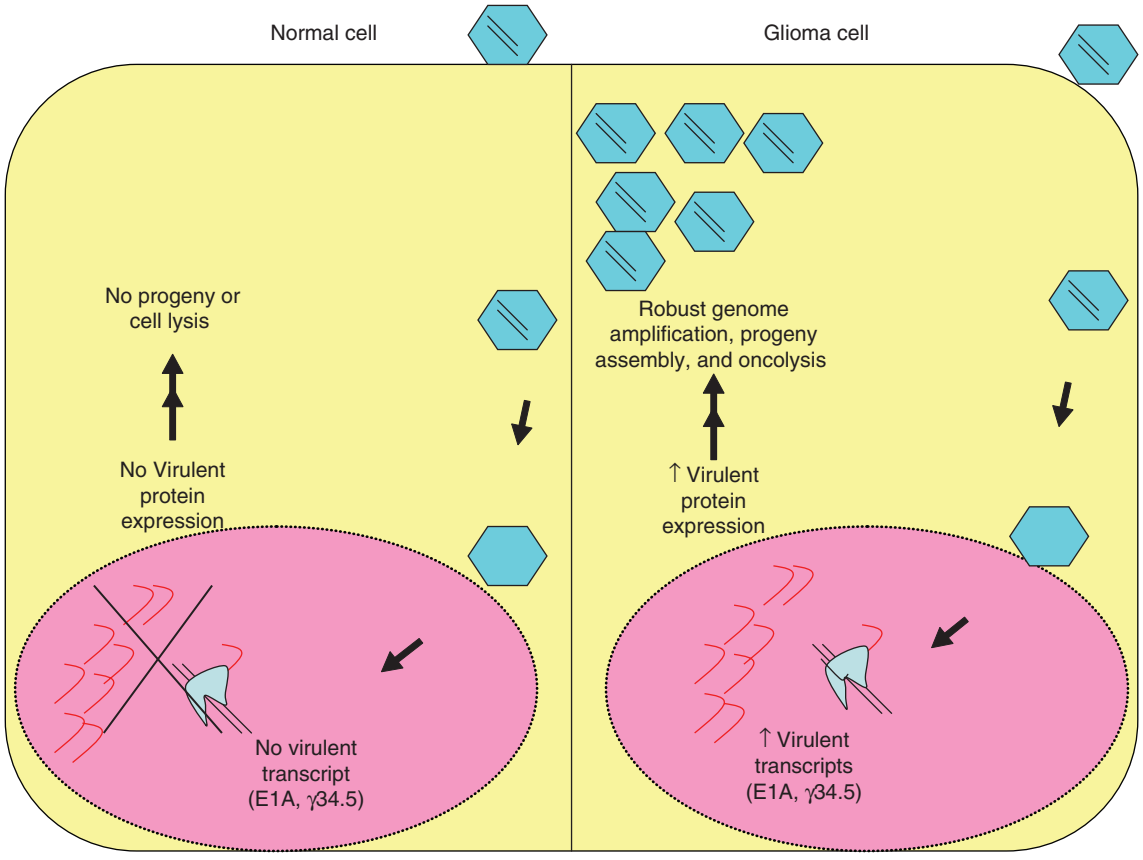

Figure 3. Schematic of transcriptional control of viral oncolysis. The replication and concomitant cell lysis of a viral vector is strengthened and made specific by incorporating tumor-specific promoters or response elements of upregulated transcription factors. These elements promote the expression of viral proteins important for robust viral replication.

3.2.3 *Transcriptional control of viral oncolysis*

A Catch-22 involved in cancer complement viral replication is that in attempting to obtain specific viral replication through deletion mutations in regions such as E1B (Ad), E1A (Ad), $\gamma_1$34.5 (HSV-1) and U_L39 (HSV-1), the most desired trait of an oncolytic vector is compromised – its toxicity. To address this problem, researchers hypothesize that transcriptional targeting of viral replication genes could serve as a means to achieve strong yet specific viral oncolysis (**Figure 3**).

The progression of all malignancies is associated with aberrant upregulation of genes involved in cell proliferation – malignant glioma is no exception. To potentiate the tumor-specificity of viral replication, researchers exploit the characteristic upregulation of genes associated with malignant progression to promote the expression of virulent genes. To this end, promoter and/or enhancer regions from upregulated genes, or response elements of highly active transcription factors, are cloned upstream of *intact* viral toxicity genes such as *E1A* and *$\gamma_1$34.5* (**Figure 3**) [70]. In this way, viral replication is robust yet localized to neoplastic foci.

Our group has demonstrated the enhanced anti-tumor effect of a conditionally replicating Ad harboring a survivin promoter upstream of the viral E1A replication protein [71]. Human survivin is a member of the inhibitor of apoptosis family of proteins (IAP), the expression of which correlates to increased recurrence and decreased susceptibility to chemotherapy and radiotherapy in patients afflicted with malignant glioma [72-75]. Members of our group reasoned that the expression patterns of this protein were prevalent enough to serve as a feasible system for controlling adenoviral-mediated oncolysis. It was demonstrated that this vector sufficiently provided robust and selective oncolysis both *in vitro* and *in vivo*, when compared to wild type 5 Ad. This vector also prevented tumor progression, decreased microvessel density and increased the survival of athymic mice bearing intracranial tumors, when compared to wild type Ad5.

Transcriptional control of viral replication has also been undertaken to increase viral burst size (oncolysis) of HSV-1 vectors. In a recent study by Kambara and colleagues, the authors demonstrate that strong yet safe oncolysis is feasible when they cloned the promoter for

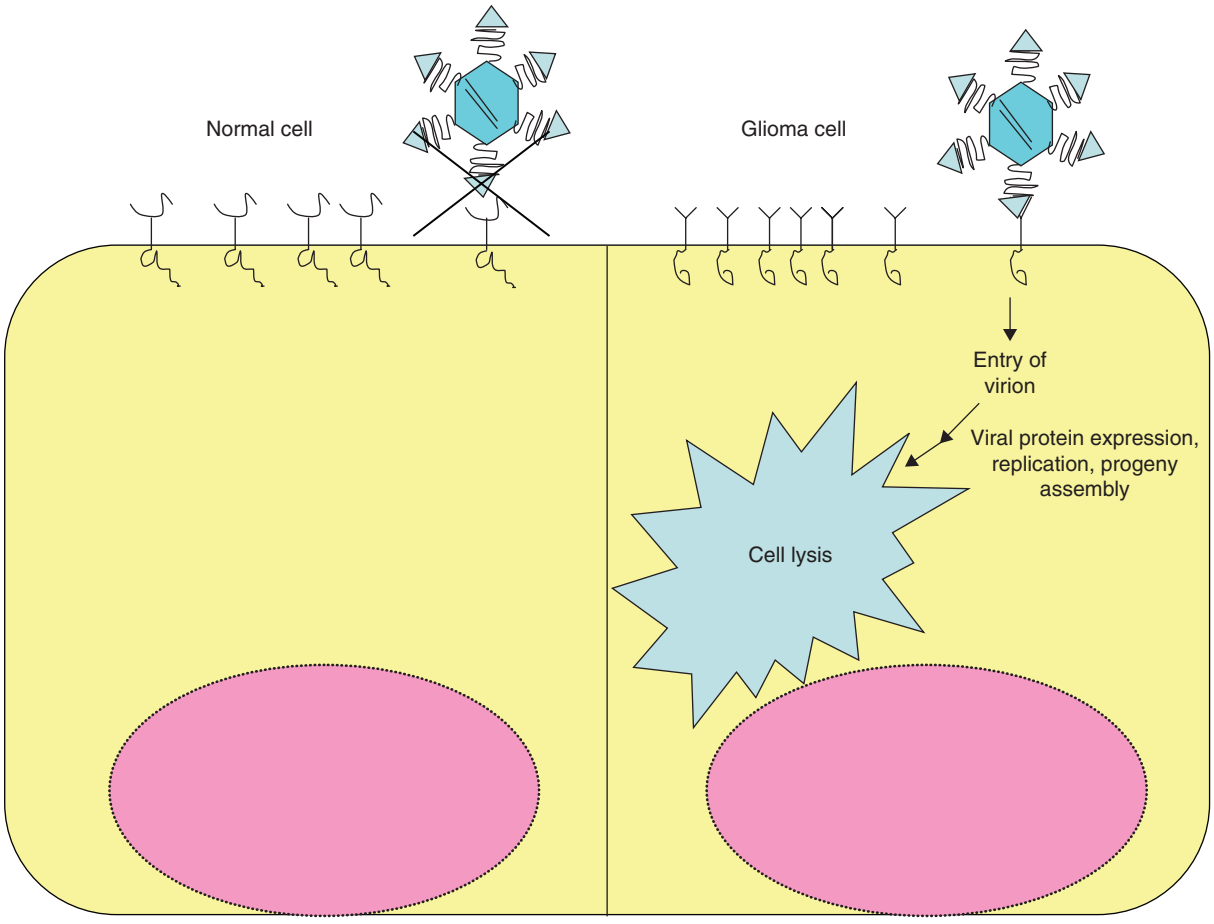

Figure 4. Vector retargeting to glioma-specific receptors. Viral vectors are engineered to target receptors that are preferentially expressed at high densities on glioma cell surfaces. A viral vector's protein capsid can be modified to include receptor-specific ligands, high-affinity motifs, or the binding site of another viral serotype.

the nestin protein upstream of the *$\gamma_1$34.5* gene [76]. Nestin is a class VI intermediate filament protein that serves as a marker of neuroepithelial precursor cells during neuronal embryogenesis [76]. The expression of the nestin intermediate filament is silenced in normal adult brain but is active in primary CNS malignancies [77]. Investigators from Kambara *et al.* showed that nestin-mediated expression of $\gamma_1$34.5 provided significantly greater glioma toxicity than a parent vector, rHsvQ1, an HSV-1 vector genetically similar in composition to the clinically tested G207. A striking finding was that this vector shared a similar safety profile to rHsvQ1 when tested *in vivo*; and, when administered to athymic mice at the time of symptom manifestation, improved survival time by 50% longer after the time of administration, when compared to rHsvQ1.

3.3 Transductional control: retargeting vectors to glioma tissue

To combat the low tumor transduction encountered by investigators in clinical trials, many researchers have attempted to enhance viral entry and replication to neoplastic tissue by retargeting vectors to alternate receptors (**Figure** 4). A common approach to viral re-targeting is to identify a receptor over-expressed on tumor tissues yet under-expressed on surrounding brain tissue. Typically, these receptors may be linked to infiltration, adhesion, or other malignant properties that manifest atypical surface phenotypes [78]. One can improve viral transduction by modifying the virion capsid to include receptor-specific ligands or motifs, generating a chimeric vector, or by substituting the surface binding site with the binding region of another viral vector, a strategy often referred to as vector pseudotyping [79,80]. These maneuvers effectively re-target viral vectors to glioma tissue and allow for more efficient and tumor-specific viral internalization (**Figure** 4).

With regard to Ad, our group and others have shown the efficacy of this approach through the use of Ad5 vectors containing a polylysine motif, RGD (Arg-Gly-Asp) motif and pseudotype modifications. The pk7 polylysine motif consists of a tandem array of positively charged lysine

residues incorporated into the C-terminal region of the Ad5 knob. This incorporation targets heparan sulfate proteoglycans over-expressed on gliomas in addition to other polyanionic receptors on the cell surface [81]. We have shown that this modification is superior to the more commonly used incorporations [82]. The RGD motif has become a widely used modification which renders Ad with enhanced glioma cell entry. It is believed that superior entry is achieved via the ability of the RGD motif to recognize the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins commonly over-expressed on the cell surface of many gliomas [82]. Pseudotype vectors have been reported to expand the susceptibility of glioma to viral infection [83]. Of late, researchers have shown the efficacy of substituting the Ad5 fiber and/or knob domains of group B Ad serotypes 3, 16 and 35 [80,84-88]. For instance, Hoffman *et al.*, in a recent study, demonstrated that an Ad5 vector containing the human Ad serotype 35 fiber and knob domains conferred enhanced transduction and anti-tumor effect when compared to its wild type Ad5 counterpart [87].

As far as HSV-1 transductional modifications are concerned, Roizman and co-workers have laid the groundwork for HSV-1 retargeting strategy. Compared to Ad5, HSV-1 transductional modifications are considerably more challenging because HSV-1's outer structure contains functional envelope and glycoprotein constituents [89,90]. Nevertheless, Kamiyama and Zhou from the Roizman group have shown that HSV-1 vectors can be rendered gliomatropic by incorporating glioma-specific receptor ligands, such as the N-terminal fragment of the urokinase-type plasminogen activator (uPA) to target the cognate receptor uPAR, and the interleukin-13 ligand to target the IL13-α2 receptor [89,90]. uPA upregulation has been identified on very aggressive forms of malignant glioma and has become a target in malignant glioma therapy because its activity is implicated in glioma infiltration via ECM degradation, cell adhesion and chemotaxis [91-96]. The IL13-α2 chain is a monomeric IL-13L receptor expressed on malignant glioma tissue and while its role in signal transduction remains a topic of debate, it is thought to be a potential therapeutic target for malignant glioma [97-99]. The authors show in these studies that retargeting of HSV-1 can be achieved by incorporation of these ligand peptides into the glycoprotein D region of the viral envelope. A relevant preclinical evaluation of these vectors has yet to be carried out.

3.4 Host immunity and oncolytic efficacy

Several proponents of virotherapy see vector-mediated immune activation as an added antitumor benefit of such systems. Some researchers, however, suggest that the immune microenvironment present in malignant glioma decreases the oncolytic effect by local clearance of the virus [25]. In a series of studies, the collaborative research of the Chiocca and Kaur groups have shown the relevance of early host immunity in hindering viral-mediated oncolysis. Members from these groups together have demonstrated that oncolytic vector administration into both mice and rats bearing intracranial gliomas incites rapid viral clearance (6 and 72 h after vector administration) by the innate arm of the immune system response [100-104]. In a number of studies over the course of a few years, authors from these groups have drawn a link between vector administration and increased vascular permeability, increased levels of IFN-γ, infiltration by NK cells, $CD68^+$ microglia and $CD163^+$ peripheral macrophages, and $CD45^+$ leukocytes. These effects can be reversed – and thus, viral replication is restored to an extent – when rats are pretreated with immunosuppressants such as clodronate liposomes (CL) and cyclophosphamide (CPA) [100-104]. These recent studies present data indicating that oncolytic therapy may benefit when administered after depleting cellular populations that negatively affect viral burst size.

4. Monitoring the efficacy vector therapy

Paramount to the generation of vectors suitable for clinical evaluation is the development of technologies that allow for non-invasive imaging of targeted vector delivery and activity [105]. Many of the clinical trials previously mentioned suffered in part because protocols did not include techniques that allowed for the monitoring of specific tumor regions and their response to vector administration. This limitation, however, is not exclusive to gene therapy, but all treatment paradigms for malignant gliomas face this problem – radiation, chemotherapy and surgery included. High grade malignant gliomas are inherently heterogenous tumors and, as such, there is a need to discriminate the malignant regions of the tumor – ones that can be targeted by vector therapy – from necrotic or nonviable regions in addition to the tumor tissue's response to a vector therapy.

Currently, magnetic resonance imaging (MRI) represents the 'gold standard' for imaging the structural components of intracranial malignancies. The 'bread and butter' of MRI lies in its ability to decipher the magnetic environment (mostly through hydrodynamic fluid flow) of the tumor to give a contrasted image. MRI's capability centers on the existence of paramagnetic atoms (i.e., hydrogen from water, iron) in bodily molecules and their behavior in applied magnetic fields. There are contrast enhancers, in addition to the commonly used gadolinium compound, currently under research that can provide details of physiological processes in glioma and response to gene therapy. In a previously mentioned study [102], the authors imaged the *in vivo* inflammatory response to oncolytic virus using monocrystalline iron oxide nanoparticles (MION) which allowed imaging of phagocytic cells infiltrating the brain and tumor microenvironment. Iron oxide nanoparticles have also been used to enable MR imaging of neovascularization in glioma using bone marrow-derived stem cells [106].

Positron emission tomography (PET) imaging technology holds much promise for targeted gene therapy applications [107]. Currently, PET's potential for delineating heterogenous regions of a brain tumor is a function of the differential activity of specific transporters in normal or necrotic brain tissue and tumorigenic tissue. For instance, radionuclides such as [2-^{18}F-Fluoro-2-deoxy-D-glucose] ([^{18}F]FDG) and *methyl*-^{11}C-L-methionine ([^{11}C]MET) are commonly used in clinical scenarios to asses the active transport of glucose ([^{18}F]FDG) and amino acids ([^{11}C]MET) in the brain. Given their specificity for characteristically overactive metabolic pathways in cancer, radiotracers can function as surrogate markers for cell density, neovascularization, or proliferative activity [107,108]. The pathways or molecular targets that can be imaged by PET are virtually limitless, provided that: i) the molecule(s) can be radiolabeled; and ii) one has access to a cyclotron. These elegant imaging protocols can be used in conjunction with vector therapy protocols to allow for the guided targeting and subsequent measurement of a vector's activity in real time. In a representative study, Jacobs *et al.* utilized a multimodal imaging technique – often termed 'co-registration' – including MRI and several PET tracers to monitor the *in vivo* targeting and efficacy of an HSV-1 amplicon vector, HSV-*cd*IRES*tk39gfp* [108]. The activity of the vector was imaged with the 9-[4-^{18}F-fluoro-3-(hydroxymethyl)-butyl]guanine ([^{18}F]FHBG), which allowed monitoring of HSV-TK activity. While the study was not without its complications, the authors provide evidence suggesting the feasibility of such approaches.

Bioluminescence represents a versatile experimental method for the *in vivo* imaging of specific biological pathways. Bioluminescence measures photon emissions from luciferase-catalyzed reactions. Because luciferase is so prevalent a marker of gene expression in many vector systems, luciferase co-expression cassettes can be used in a variety of ways to measure glioma progression and/or the therapeutic activity of a vector. Luciferase bioluminescence has been applied for *in vivo* tracking of migrating neural stem cells to glioma and also to measure the activity of an oncolytic Ad against glioma [104,109].

5. Conclusions

Vector therapy for malignant glioma represents an attractive modality for the treatment of malignant glioma. The theory behind vector therapy paints a very promising picture of its potential in the clinical setting. However, the practical application of theory is often accompanied by unexpected and disappointing outcomes. Such was the case with first generation vector therapies in malignant glioma clinical trials. However, these setbacks have allowed scientists to refocus their attention on optimizing vector delivery to malignant brain tissue. The representative studies presented in this review are not exhaustive by any means, but they provide a comprehensive and representative illustration of what is currently being examined in the field of vector therapies for malignant glioma.

6. Expert opinion

With regard to malignant glioma, vector therapies possess vast potential for clinical application. Despite the extensive amount of research that has gone into this area, the field is still in its infancy. For a particular vector to be successful in clinical applications, it must demonstrate selective and persistent anti-tumor activity and the ability to 'seek out' diffuse tumor foci that often spawn recurrent progression, all without eliciting a response from the host immune system. Most of the research regarding vector therapy is aimed at satisfying this strict set of criterion. The research demonstrated in this review provides insight into the prevalent areas of study and give evidence strongly suggesting the feasibility of these systems once again being implemented in clinical protocols in full force. But before these new advances become applied into clinical practice, there are important factors that remain to be addressed.

6.1 Stem cell vector delivery

Since stem cells – specifically NSCs and MSCs – possess the ability to migrate to areas of malignant glioma pathology, they are very attractive candidates for targeted vector delivery. These cells represent a novel approach for vector delivery, and as such, significant questions remain regarding the molecular mechanisms underlying the tropism of these stem cells for glioma. Specifically, what molecular factors are responsible for the migration of these cells *in vivo* and from what microenvironment in malignant tissue do they originate? Is there a particular substrate(s) or 'niche(s)' present in malignant tissue where these stem cells preferentially home? What is the duration and degree of transgene expression provided by a gliomatropic stem cell and is it satisfactory enough to combat tumor recurrence in a specific manner? Of equal importance, what is the transgene(s) that provides the best anti-tumor effect? Which stem cell is more capable of delivering anti-tumor genes to malignant glioma and which is more practical? NSCs seem to be the optimal candidate for delivering vectors, but obtaining them has become the subject of much controversy, as they are isolated from human fetuses. On the other hand, MSCs are easier to obtain, and more practical for clinical-grade production and use, as they can be delivered autologously. Addressing and answering these questions will be a major focus of investigators in years to come and will be crucial in determining the use and entry of these cells into the clinical setting.

6.2 Viral vector delivery

Over the years, oncolytic therapy for malignant glioma has remained an intense area of research investigation and this trend will continue in the future. The design of a vector

with optimal specificity and toxicity will remain a primary focus of investigators. At present, the use of vectors with combinatorial modifications including transductional, transcriptional, as well as deletion mutations appears a feasible approach for obtaining a vector with a high therapeutic index [110]. Vectors including all three of these modifications will become common practice in the future; however, modifications must be made such that a vector's oncolytic capacity is not significantly compromised.

Malignant glioma represents one of the most aggressive and devastating types of neoplasms. Thus, physician scientists must adopt a 'fight fire with fire' approach when it comes to evaluating novel therapies for the treatment of this disease. It is possible that the use of viral vectors as a monotherapy for recurrent glioma will not confer significant anti-tumor effects. Very recently, viral vectors have been evaluated as adjuvants to chemotherapy and radiotherapy [111-113]. The identification of a vector that renders resistant glioma cells susceptible to chemotherapy/radiotherapy and vice versa represents an attractive paradigm for treating CNS malignancies. Combining these treatment modalities could potentially produce a synergistic anti-tumor effect leading to increases in the survival of patients diagnosed with malignant glioma.

While researchers have made significant strides to improve the rate of viral entry into glioma tissue, the issue of vector distribution remains a trying endeavor. The use of the gliomatropic stem cells discussed in this review to deliver replication-deficient vectors has been demonstrated [114,115]. Just recently, the use of the same cells to distribute oncolytic vectors has been evaluated in other cancer models, including lung and breast cancer [116,117]. Our group has demonstrated the feasibility of this approach using mesenchymal stem cells to deliver an oncolytic vector to intracranial glioma [118]. It would be ideal if it were possible to combine the selective and potent killing capacity of an oncolytic vector with the glioma-tracking capabilities of stem cells. The identification of an oncolytic vector which has little effect on stem cell mobility but high glioma toxicity is of critical importance in this approach and future research should address this issue.

The immune system response in tumor pathobiology remains a puzzling phenomena and a concentrated area of research for immunologists and gene therapists alike. It will be interesting to see how the immune system's role in vector therapy will pan out. Some perceive that viral vectors will provide the ancillary benefit of anti-tumor immune induction; conversely, some view the rapid response of the host immune system to vector therapy as something that should be countered with immunosuppressants. It is possible that both are valid assertions. An optimistic assumption making both cases compelling would be that an initial clearance of a local innate reaction could provide enough time for an oncolytic vector to establish multiple rounds of infection and adequate distribution in the diseased tissue. At this period of infection, an ample anti-viral/anti-tumor response by innate and adaptive immune arms would constitute a desirable host response to therapy. Unraveling the details of the host's immune reaction in regards to vector therapy will be concomitant with discoveries involving the host immune system's role – irrespective of vector therapy – in malignant glioma pathophysiology.

The successful application of targeted vectors for intracranial malignancies will center on the development of non-invasive *in vivo* imaging systems capable of depicting specific regions of the tumor. As stated previously, this is not something unique to gene therapy but all anti-tumor therapies for malignant glioma. The capability of imaging protocols to map out desirable targets within glioma has been difficult, considering a number of hurdles: i) malignant glioma are characteristically heterogenous neoplasms; ii) the functional basis of NMR – magnetism – renders it limited in specificity and sensitivity in imaging a desired target; iii) with energy consumption being highest in the brain, the ability of certain radiotracers used in PET, by themselves, to decipher distinct tumorigenic regions from normal brain is variable; iv) many of these assays require access to very expensive technologies; v) bioluminescence is currently only relevant in experimental and not clinical protocols. That being said, the development of imaging systems for therapeutic application is a major endeavor in itself; yet its advancement is crucial to the furthering of vector therapies. With the advent of more sophisticated visual tracers and genetic engineering techniques [119], researchers will be able to assay *in vivo* if their engineered vectors are capable of targeting the intended pathway. Ideally, the vector delivering the damaging blow to a tumor would also possess regions in its genome that can directly co-operate (by physical or chemical means) with an imaging modality. In some respects this may prove more feasible than with other therapies such as radiation and chemotherapy. The limitations would be the size allowed for inclusion of a marker gene in a particular vector, the short half-life of a marker and a potential lack of specificity in the interaction between imaging tracer and the biomarker of the vector. Adenovirus and HSV-1 vectors have potential in these areas because they provide stable and predictable gene expression patterns. Successful application requires knowledge of both the kinetics of an imaging tracer's activity in tumor tissue and the kinetics of a vector's gene expression.

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