Synthetic and systems biology principles in the design of programmable oncolytic virus immunotherapies for glioblastoma multiforme

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Abstract

Oncolytic viruses (OVs) are a class of immunotherapeutic agents with promising preclinical results for the treatment of glioblastoma multiforme (GBM) but limited success in recent clinical trials. Advanced bioengineering principles from disciplines like synthetic and systems biology are needed to overcome the current challenges faced in developing effective OV-based immunotherapies for GBMs, including off-target effects and poor clinical responses. Synthetic biology is an emerging field that focuses on the development of synthetic DNA constructs that encode networks of genes and proteins (synthetic genetic circuits) to perform novel functions, whereas systems biology is an analytic framework that enables the study of complex interactions between host pathways and these synthetic genetic circuits. In this review, we summarize synthetic and systems biology concepts for developing programmable, logic-based OVs to treat GBMs. Programmable OVs can increase selectivity for tumor cells and enhance the local immunological response using synthetic genetic circuits. Here we discuss key principles for developing...
programmable OV-based immunotherapies including how to (1) select an appropriate chassis—a vector that carries a synthetic genetic circuit—and (2) design a synthetic genetic circuit that can be programmed to sense key signals in the GBM microenvironment and trigger release of a therapeutic payload. To illustrate these principles, we include some original laboratory data highlighting the need for systems biology studies as well as some preliminary network analyses in preparation for synthetic biology applications. Examples from the literature of state-of-the-art synthetic genetic circuits that can be packaged into leading candidate OV chasses are also surveyed and discussed.

Keywords
synthetic biology; systems biology; oncolytic virus; cancer immunotherapy; glioblastoma multiforme

1. Synthetic and systems biology approaches in glioblastoma multiforme

Glioblastoma multiforme (GBM) is the most common and lethal malignancy of the central nervous system (CNS). This devastating disease has a median overall survival of 3 months from time of diagnosis in untreated patients\(^1\). Despite the significant cost and morbidity associated with standard of care surgical resection combined with adjuvant chemotherapy and radiation, median life expectancy is only extended to about 14 months and the disease remains almost uniformly fatal\(^2,3\). This is the best we can do for patients after hundreds of clinical trials over several decades. Immunotherapies are breakthrough options for many cancer patients but, despite promising safety profiles, are not currently effective for those suffering from GBM\(^4\).

Oncolytic viruses (OVs) are a class of immunotherapeutic agents with an FDA-approved treatment for melanoma—a solid tumor\(^5\). OVs work by directly lysing tumor cells, which then release tumor antigens in the context of danger signals—both damage-associated (DAMPs) and pathogen-associated molecular patterns (PAMPs)—that elicit antitumor immunity (Figure 1A)\(^6\). Since 1991, many attempts to use OVs in GBM have had limited success. Over 20 clinical trials of 7 different OVs did not translate encouraging preclinical results to GBM patients\(^7\). The root causes of these failures can be traced to failed oncotropism—despite neurosurgical delivery—and oncolysis mechanisms\(^7\). Therefore, advanced bioengineering methods are necessary to design and implement better armaments.

Synthetic biology involves the creation and manipulation of biological systems using rational, modular design principles from electrical engineering, computer science, and related disciplines\(^8\). The sense-compute-actuate framework (Figure 1B)\(^9\) is a guiding design principle in synthetic biology where long stretches of synthetic DNA constructs that encode networks of genes and proteins (synthetic genetic circuits) are designed to sense stimuli of interest, compute which environmental state a cell is in based on a permutation of stimuli, and actuate a response accordingly. In November 2019, the first living medicine containing a synthetic genetic circuit entered Phase 1 clinical trials in the form of a bacteria-based cancer immunotherapy\(^10\). Such engineered genetic circuits are now primed to be packaged into OVs.
and programmed to coordinate local tissue responses, with preclinical studies demonstrating enhanced (1) tumor cell targeting and (2) generation of antitumor immunity.

Systems biology complements synthetic biology by enabling complex design and analysis of genetic circuits. Tumor microenvironment (TME) cell states can be defined by using single cell multiomics datasets such as RNA sequencing (RNA-seq) and mass cytometry. These high dimensional datasets are amenable to machine learning\(^\text{11}\) and network-based classification\(^\text{12}\). Computation identifies biological features unique to glioma cells\(^\text{13}\), elucidate pathways orthogonal to genetic circuit designs, and help prioritize OV-based approaches\(^\text{14}\). After the synthetic OVs are constructed and tested, further systems analyses ensure that the OVs have predictable effects on the cells and the greater TME, statistically accounting for complex stochastic behavior. This knowledge is fed back into the design of improved, next generation OV genetic circuits (Figure 1C).

2. Selection and delivery of OV chasses

Selection of a chassis—a viral vector used to carry a synthetic genetic circuit in this case—is essential to the design of any synthetic biology system. In terms of OVs, given that we do not yet have the technology to design a virus de novo, this means identifying a naturally occurring virus that can be engineered to selectively lyse tumor cells. The essential parameters to consider are payload capacity, tropism, life cycle, immunogenicity, and tumor delivery. These considerations are just starting points since synthetic biology can be used to address some limitations inherent to the wild-type chassis.

The chassis needs to be able to accommodate sufficient genetic material to produce a functional circuit or, in the case of a combination OV therapeutic approach, a circuit component. Ideally, it should also avoid targeting sensitive host cells at baseline while preferentially entering and replicating in tumor cells. If not, then the chassis needs to be highly amenable to engineering of its entry and metabolic tropisms. To generate antitumor immunity, the innate immune system must be activated at some point during OV replication. This awakens the adaptive immune system to precisely target all cancerous cells, both infected and uninfected\(^\text{15}\). If the patient has been exposed to the viral chassis before, either by infection or vaccination, then this may limit the efficacy of the OV. Host genome integration (e.g. lentivirus) may be advantageous for persistent anti-tumor effects but comes with the risk of genetic damage and chronic latent infection\(^\text{16}\). Ultimately, however, the immune system must be able to ultimately clear the OV infection, particularly in the CNS to prevent chronic inflammation and its sequelae.

2.1 Neurotropic viruses

A chassis needs to target glioma cells, which arise from healthy cells of the brain parenchyma and share many cell surface and metabolic features, so naturally-occurring neurotropic viruses are the obvious starting point.

2.1.1 Herpes simplex virus type 1—Herpes simplex virus type 1 (HSV-1) is an enveloped virus with a linear 152 kilobase (Kb) double-stranded DNA (dsDNA) genome\(^\text{17}\). It is capable of carrying large payloads—upwards of 20 Kb—making it particularly
attractive for synthetic biology applications. It is the first and only OV that has garnered FDA-approval with talimogene laherparepvec, which has been engineered extensively to target melanoma\textsuperscript{18}. HSV-1 against GBM was also the first proposed OV with experimental success in a murine model\textsuperscript{19}. Therefore HSV-1 is particularly promising as a treatment for GBM.

The high prevalence of HSV-1 infection in humans, however, means that preexisting immunity (e.g. neutralizing antibodies) poses a barrier to its application as an effective OV. Modulating this OV-host immune interaction is a possibility with synthetic gene circuits\textsuperscript{20}. Additionally, several molecular features of GBM have been identified that influence the efficacy of HSV-1 OV \textit{in vitro} and \textit{in vivo}, including the integrin ligand cysteine-rich 61 protein (CCN1) found in the extracellular matrix of aggressive gliomas\textsuperscript{21}. Pathway analysis of HSV-1-resistant GBM has informed the design of improved OV, such as HSV-1 armed with a vasculostatin payload to abrogate the integrin signaling effects of CCN1 and thus enhance oncolysis\textsuperscript{22}.

Integration of multiple pathways gives rise to networks that offer a systems-level view of OV resistance mechanisms. Using our systems biology network analysis tools\textsuperscript{12}, our group has recently identified key protein-protein interaction networks that play a role in the cellular response to HSV-1\textsuperscript{13}. In particular, we have identified a prioritized subnetwork associated with CCN1-high expressing LN229 glioma cells (Figure 2A). Network edges (Figure 2B) and nodes are currently being evaluated for their utility in this GBM subtype. Genetic circuits\textsuperscript{23} that sense and exploit these networks could enhance the efficacy of HSV-1 OV—for example, using a simple Boolean AND gate in which two repressors sense distinct input RNAs and output a pro-apoptotic or pro-inflammatory molecule only if both RNAs are present (Figure 2C).

2.1.2 Poliovirus—Poliovirus, a non-enveloped 7.5 Kb positive-strand RNA virus, enters cells via CD155. A recombinant non-neuropathogenic polio-rhinovirus chimera (PVS-RIPO) has demonstrated some clinical efficacy and safety in early clinical trials\textsuperscript{24}. It infects both glioma cells for direct oncolysis and antigen presenting cells to stimulate adaptive antitumor immunity. Therefore it is suitable for delivering genetic circuit components that may signal between these cell types. Whole-genome poliovirus synthesis enables significant build control despite limited payload capacity\textsuperscript{25}.

2.1.3 Other neurotropic viruses—Vesicular stomatitis virus (VSV), enveloped with an 11 Kb non-segmented, negative-sense RNA genome, has been able to destroy GBM cells with some success\textsuperscript{26} but has thus far proven prohibitively neurotoxic. Synthetic circuitry may fine-tune VSV neuroimmune interactions for better outcomes\textsuperscript{27}. Zika, a positive-sense RNA virus, enters glia via the receptor tyrosine kinase AXL and has activity against GBM stem cells\textsuperscript{28}.

All of these OVs, however, run the risk of chronic neuroinflammation—potentially leading to neurodegeneration—due to their inherent neurotropism. Using attenuated, pseudotyped, chimeric, or alternate serotype strains of these OVs have not fully eliminated adverse effects nor sufficiently increased efficacy against GBM.
2.2 Non-neurotropic viruses

There is an opportunity to use non-neurotropic chasses to engineer safer OV’s using synthetic biology.

2.2.1 Measles virus—Measles virus is an enveloped negative-sense RNA virus that islymphotropic. Measles enters the cell through interaction with CD46, which isoverexpressed by glioma cells with stem-cell-like properties. Numerous strains of measlesdemonstrate efficacy against GBM. This efficacy can be improved with adjuvantradiotherapy and immune checkpoint blockade, suggesting mechanistic opportunities for synthetic gene circuits to enhance synergy. Systems biology has been used to predict GBMpermissiveness of measles OV, yielding a naïve Bayes machine learning-based classifier,which could inform improved designs using synthetic biology. Despite being non-neurotropic, there is risk of neurological sequelae due to chronic neuroinflammation, such as subacute sclerosing panencephalitis.

2.2.2 Vaccinia virus—Vaccinia virus is an enveloped virus with a linear 190 Kb dsDNA genome with about 220 protein-coding genes. It was the first safe and effective vaccine used in humans, ultimately eradicating the smallpox pandemic. The large, complex structure of vaccinia virus lends itself to extensive genetic modification. Vaccinia OVs engineered with tumor suppressor p53, immunomodulatory granulocyte-macrophage colony stimulating factor (GM-CSF), differentiation factor bone morphogenetic protein-4 (BMP4) and suicide gene FCU have shown some preclinical success against GBM.

2.2.3 Adenovirus—Adenovirus is a particularly strong candidate OV for GBM because of its negligible neurotropism and associated neurotoxicity. Its linear, 36 Kb double-stranded DNA genome—which yields myriad spliced transcripts—is amenable to bioengineering using robust synthetic biology methods. The non-enveloped adenovirus serotype 5 (Ad5) enters cells natively via the coxsackievirus and adenovirus receptor. For safety, a 24-bp deletion in E1A (Δ24) ensures that Ad5-Δ24 replicates only in cells lacking the retinoblastoma tumor suppressor protein (pRb), common in GBM but not in other CNS cells. Arming Ad5 with RGD-4C or GITRL ligands enhances glioma entry or anti-glioma immunity, while synthetic riboswitches enable external regulation for safety. It is a highly promising OV given its anti-GBM effects alone or in combination therapies, both in animal models and in recent clinical trials as DNX-2401 (tasadenoturev). Innate resistance to adenovirus poses a significant challenge because OV permissiveness in GBM is the essential first step to success as an immunotherapy. Elucidating complex tumor-OV interactions at the systems level can identify immuno-resistance mechanisms. We have begun to survey the Mayo Clinic Brain Tumor Patient-Derived Xenograft (PDX) National Resource for GBM responses to Ad5-Δ24. The resource currently has 92 GBM PDX models with associated data on the clinical phenotype, as well as growth characteristics, invasiveness, molecular subtype, and tissue microarray data. We have found a wide array of dose- and time-dependent responses to the Ad5-Δ24 (Figure 3A-B). Our previous systems biology analyses suggest that these variable responses could be due to network effects.
Targeting network edges and nodes critical to resistance using genetic circuits (as in Figure 2A-C for HSV-1) could convert resistant GBM to permissive.

2.2.4 Other non-neurotropic viruses—Reovirus, a replication-competent dsRNA virus that exploits Ras-signaling, has shown clinical safety and efficacy in glioma patients\textsuperscript{49} and synergizes with checkpoint blockade\textsuperscript{50}. Preclinical data supports the use of Sindbis virus in GBM but clinical development has languished\textsuperscript{51}.

These and other potentially useful chasses (summarized in Figure 4), along with the current state of clinical trials for gliomas, are presented in detail by Martikainen, et al.\textsuperscript{52} and Eissa, et al.\textsuperscript{53}. While many show some promise, no candidates presently offer more than incrementally better outcomes for our patients. Major hurdles include susceptibility to innate antiviral immunity\textsuperscript{54}, tumor escape from adaptive immunity\textsuperscript{55}, and inflammatory neurodegeneration\textsuperscript{56}. Arming OVs with synthetic genetic circuits could offer better control over the treatment and may lead to drastically better results.

2.3 Delivery approaches

Most OV clinical trials for gliomas have relied on neurosurgical delivery using direct intratumoral or intraparenchymal bolus injection. This effectively bypasses the blood-brain barrier, overcomes interstitial hypertension, and reduces off-target effects, but increases risks of stroke, infection, and mass effect, as well as costs. Minimally-invasive procedures, including local convection-enhanced delivery through a transcranial cannula\textsuperscript{57} and regional intra-arterial delivery via carotid or vertebral catheterization\textsuperscript{58}, may be advantageous.

Systemic intravenous delivery would be ideal in terms of patient experience and neurosurgical utilization. Pseudotyping and polymer coating have had limited success in achieving this goal, largely because the OVs are rapidly cleared from circulation\textsuperscript{59}. Loading OVs into cellular carriers—reovirus into monocytes \textit{in situ}\textsuperscript{60} or measles into mesenchymal stem cells \textit{ex vivo}\textsuperscript{61} for example—appears most promising. This approach expands the chassis to include the cell-based delivery vehicle for purposes of genetic circuit design.

3. Genetic circuit design for programmable OV-based immunotherapy

Synthetic genetic circuits can be packaged into OVs and used to increase the selectivity of viruses to tumor cells, as well as enhance the local immunotherapeutic response. Here we illustrate several genetic circuit design principles based on a programmable OV recently reported in the literature and apply these principles to designing OV-based immunotherapies for GBMs.

3.1 General genetic circuit design principles

Results from Huang, et al.\textsuperscript{62} on the efficacy of a programmable OV-based immunotherapy in mice effectively demonstrate several principles for designing synthetic genetic circuits. The investigators developed an oncolytic adenovirus programmed by a 6.5 Kb synthetic gene circuit to selectively replicate and release immune mediators in hepatocellular carcinoma cells. Using synthetic biology principles, they created a genetic circuit with interchangeable cancer-selective promoters, micro RNA (miRNA) target sites to detect distinct miRNA
profiles of cancer cells, and genes that encode immune mediators like interleukin-2 (IL-2), GM-CSF, or single-chain variable fragments (scFvs) against either programmed death-1 (PD-1) or programmed death-ligand 1 (PD-L1).

The overall design of the circuit involves a promoter selectively activated in hepatocellular carcinoma cells (pCancer) that drives expression of a potent transcriptional activator (Gal4-VP16) which in turn drives expression of two mutually inhibitory repressors, Rep-a and Rep-b. The expression of these repressors is inhibited by miRNAs, miR-a and miR-b, respectively for added selectivity. miR-a is a stand-in for any miRNA that is high in normal cells and low in cancer cells, whereas miR-b is a miRNA that is high in cancer cells and low in normal cells. The expression of Rep-a is coupled using autocatalytic linker proteins (L) to protein E1A which increases adenoviral replication and an immune effector of choice (IL-2, GM-CSF, scFvs against PD-1 or PD-L1) that stimulates the host immune response. Autocatalytic linker proteins effectively reduce the amount of DNA required to transcribe three separate proteins by combining them into one therapeutic payload.

The complex logic encoded by their genetic circuit achieves increased selectivity, as well an enhanced host immune response. In normal cells, where miR-a is high, miR-b is low, and pCancer has basal activity, expression of the genetic circuit payload of E1A linked to an immune effector linked to Rep-a is low. Additionally, the activity of Rep-b and miR-a further reduces payload expression below basal levels, limiting replication of OV in normal cells. In cancer cells, where miR-a is low, miR-b is high, and pCancer has high activity, there will be high expression of the genetic circuit payload. This effectively limits expression of the therapeutic payload to tumor cells.

Oncolytic adenoviruses were packaged with the synthetic genetic circuit and injected intratumorally into immune competent mice who were xenografted with hepatocellular carcinoma cells. All mice (n = 10) treated twice in one week with $1 \times 10^9$ viral particles containing synthetic genetic circuits expressing GM-CSF and scFvs against PD-1 eliminated tumors within 33 days. These are promising results of a programmable OV-based immunotherapy in a validated preclinical model for hepatocellular carcinoma. Their modular circuit design enables rapid translation to other cancers including GBM (Figures 5A-C).

### 3.2 Genetic circuit design principles specific for treating GBMs

Several design principles can be used to guide the creation of other advanced synthetic genetic circuits to program precision OV-based immunotherapies for GBMs.

#### 3.2.1 Genetic circuit design elements

**Selectively activated promoters.** Promoters that drive expression of the genetic circuit can be chosen so that they are selectively activated in brain tissue or tumor cells. This serves as a design feature that provides an initial protection against off-target effects of the viral therapy. For example, the neuron-specific enolase promoter can restrict OV expression to the brain. Other promoters like the telomerase reverse transcriptase (hTERT), glial fibrillary astrocyte protein (GFAP), and nestin have been shown to have selective, increased expression in glioma cell lines. Radiosensitive promoters like the survivin promoter which have...
increased expression in cells that are exposed to radiation can be used to selectively express the genetic circuit in irradiated GBMs.

**Binding sites for differentially expressed miRNA.** Binding sites for miRNA that are differentially expressed in GBMs can be used to enhance the selectivity of OV-based immunotherapy. Adding these binding sites to genes encoded by the genetic circuits can allow for selective expression of the therapeutic payload in cells with a particular miRNA profile. Several miRNA have been identified to be differentially expressed in GBM including miR-21, miR-93, miR-196, and miR-335, which have been shown to be upregulated, and miR-7, miR-34a, and miR-124a which have been shown to be downregulated. An example of how RNA binding sites can be used to create genetic circuits that perform simple logic operations like the AND gate is shown in Figure 2C.

**Pro-apoptotic and pro-inflammatory therapeutic payloads.** Several pro-apoptotic and pro-inflammatory genes can be selected as the therapeutic payload expressed by the genetic circuit in order to enhance the local response to the OV-based immunotherapy. Pro-apoptotic genes like secreted tumor necrosis factor-related apoptosis-inducing ligand (sTRAIL) and hBax can enhance tumor killing. Similarly, pro-inflammatory genes like IL-2, GM-CSF, scFvs against PD-1 or PD-L1 can promote local immune responses to GBM (Table 1, 2).

### 3.2.2 Other genetic circuit design considerations

**Optimizing genetic circuit size.** The size of genetic circuits used to program OVs is fundamentally limited by the quantity of DNA that can be packaged, which ranges from 4 to 15 Kb depending on the viral vector chosen. Recently developed modular DNA assembly techniques that take advantage of Golden Gate and Gibson assembly reactions have been shown to efficiently construct multi-gene expression vectors of reasonable construct size that can be packaged into a lentivirus and integrated into the genome. Moreover, there are several design strategies that may be used to optimize genetic circuit size. For example, autocatalytic linker proteins may be used to produce multiple proteins from one RNA transcript. Minimal promoter elements containing only the essential promoter sequences required for transcription to take place can be used instead of natural mammalian promoter sequences, which are often large.

**Orthogonality with host pathways.** Orthogonality is an important design principle which entails using components of a genetic circuit that operate predominantly at specified sites in the genetic circuit with minimal off-target effects. For example, Huang, et al. had used an activator (Gal4-VP16) that binds to Gal4 sites from the yeast genome and has minimal interactions with the host genome (Figure 5C). Therefore, their activator primarily interacted with their synthetic genetic circuit and was orthogonal to the host genome. If the activator was not orthogonal, an oncogene might have been activated instead, thus defeating the purpose of the genetic circuit.

**Mitigating transcriptional noise.** Expression of the therapeutic payload by the genetic circuit must be resistant to transcriptional noise so as to minimize off-target effects of the therapy. Transcriptional noise is a reality of biological systems and can lead to low level,
basal expression of the therapeutic payload in healthy cells. Network motifs like mutual repression can mitigate the effect of transcriptional noise and enhance the steepness between ON and OFF states of the genetic circuit\(^ {67,75}\). Incorporating these network motifs into any genetic circuits designed for OV-based immunotherapy is essential for ensuring safety in humans.

4. Discussion and conclusion

Synthetic and systems biology are ushering the way for novel rationally designed therapeutics that respect biological complexity. In this review, we have summarized these concepts as applied to developing programmable OV-based immunotherapies for GBMs. This approach may be limited by genetic circuit size, incomplete host network orthogonality, OV replication fidelity, and an inability of quasispecies to coevolve with GBM cells over time. OVs successfully programmed with a synthetic genetic circuit, however, have the benefits of increased selectivity to tumor cells, enhanced local response due to the release of customizable therapeutic payloads, and a modular design that allows for rapid prototyping of different miRNA binding sites and therapeutic payloads.

As we have described above, any programmable OV-based immunotherapy requires design of (1) a chassis vector with delivery vehicle and (2) a synthetic genetic circuit that encodes a network of molecular effectors that sense the tumor microenvironment and trigger release of a therapeutic payload. Chasses include both neurotropic and non-neurotropic viruses with different genome sizes and natural selectivity for tumor cells. Synthetic genetic circuits can be designed in a modular fashion with selectively activated promoters only active in GBM cells, RNA binding sites that differentiate GBM cells from healthy tissue, and customizable therapeutic payloads that release pro-apoptotic and pro-inflammatory factors.

Programmable circuits represent the natural next step in the evolution of OV immunotherapies for GBM. Promising preclinical results have been described for programmable OVs designed using synthetic biology principles. We hope that the principles outlined here will help guide the additional preclinical research development required to bring this exciting new technology to GBM patients in the near future.

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Figure 1.
(A) Mechanisms of OV immunotherapy for GBM. (B) Sense-compute-actuate framework of programmable OV immunotherapy for GBM. (C) Design-build-test-analyze development cycle of synthetic OVs for GBM blends systems and synthetic biology.
Figure 2.
(A) NetDecoder prioritized network infers salient protein-protein interactions (PPIs) in expression microarray data from LN229 human GBM cells depending on the state of CCN1 expression, predictive of HSV-1 OV resistance. PPIs are represented at edges and nodes with higher (red) and lower (blue) flows under CCN1\textsuperscript{high} state versus the CCN1\textsuperscript{low} control. Nodes consist of sources (diamonds), routers (circles), and sinks (squares). (B) PPIs are prioritized based on the CCN1\textsuperscript{high} state (yellow). Edge flow differences between these and the CCN1\textsuperscript{low} control state (blue) are ranked in order, increasing from left to right on the histogram. Each edge ID corresponds to a known PPI. (C) Repressor-based Boolean logic can be coded in genetic circuit to sense GBM (RNA-a) and OV resistance (RNA-b) network states and actuate gene expression that triggers immunogenic cell death. A prototype AND gate—with two inputs (Repressors 1 and 2) and one output (Actuator)—is shown for illustration.
Figure 3.
Representative viabilities of GBM PDX neurospheres (Sp-GBMs) after treatment with Ad5-Δ24 OV at five multiplicities of infection (MOIs) after (A) 2 and (B) 8 days. Sp-GBMs were cultured ex vivo in Gibco StemPro neural stem cell serum free medium. Cell viability was assessed using the Promega CellTiter-Blue reagent, which measures the metabolic capacity of the Sp-GBM cells to convert resazurin redox dye into resorufin. MOI is defined as 293A plaque-forming units (PFUs) per Sp-GBM cell. Error bars represent the standard error of the mean for biological triplicates.
Figure 4.
Timeline of key candidate OV chases by year first described in GBM preclinical studies. First OV strain described in the literature is shown in parentheses.
Figure 5.
Development of an oncolytic adenoviral therapy programmed with a synthetic genetic circuit to selectively replicate and express immune mediators in tumor cells. Binary truth table (A) indicates target level of each circuit element in host cells, with 0 = low and 1 = high. Design of circuit logic is schematized in (B) and build strategy is shown in (C). pCancer = cancer selective promoter; pBasal = basal promoter; E1A = adenovirus early region 1A replication protein; Activator = Gal4-VP16 transcriptional activator; Actuator = immune effector protein (IL-2, GM-CSF, scFvs against PD-1 or PD-L1); L = linker protein; Rep-a/Rep-b = Repressor a/b.
Table 1.
Genetic circuit sensor elements for creating programmable OV therapies for GBMs.

| Design Element                        | Characterization Notes                                      |
|---------------------------------------|-------------------------------------------------------------|
| Selectively activated promoters       |                                                             |
| Neuron-specific enolase (NSE)         | Activity restricted to brain, mostly neurons\(^ {62} \)      |
| Telomerase reverse transcriptase (hTERT) | Activity in U251 and T98G glioma cell lines\(^ {63} \)   |
| Glial fibrillary astrocyte protein (GFAP) | Activity in U251 and T98G glioma cell lines\(^ {63} \) |
| Survivin                              | Activity increases after irradiation\(^ {65} \)               |
| Nestin                                | Activity restricted to glioma cell lines\(^ {64} \)           |
| Binding sites for differentially expressed miRNA |                                          |
| miR-21                                | Upregulated in GBM cells, associated with increased proliferation\(^ {66} \) |
| miR-93                                | Upregulated in GBM cells, associated with tumor invasion\(^ {66} \) |
| miR-196                               | Upregulated in GBM cells, associated with increased proliferation\(^ {66} \) |
| miR-335                               | Upregulated in GBM cells, associated with increased proliferation\(^ {66} \) |
| miR-7                                 | Downregulated in GBM cells, associated with greater epidermal growth factor production (EGFR) production\(^ {66} \) |
| miR-34a                               | Downregulated in GBM cells, associated with decreased p53\(^ {66} \) |
| miR-124a                              | Downregulated in GBM cells, normal in neurons\(^ {66} \); limited off-target effects in oncolytic HSV\(^ {67} \) |
Table 2.
Genetic circuit actuator elements for creating programmable OV therapies for GBMs.

| Genetic Circuit Actuators | Characterization Notes |
|---------------------------|------------------------|
| **Design Element**        | **Pro-apoptotic payloads** |
| Secreted tumor necrosis factor-related apoptosis-inducing ligand (sTRAIL) | Promotes apoptosis via TNF family receptor signaling\(^{62}\) |
| hBax | Promotes apoptosis via release of cytochrome C from mitochondria\(^{69}\) |
| **Pro-inflammatory payloads** | |
| Interleukin (IL)-2 | Promotes inflammatory response by stimulating T cell proliferation\(^{61}\) |
| Granulocyte macrophage colony stimulating factor (GM-CSF) | Promotes inflammatory response by stimulating immune cell proliferation\(^{61}\) |
| Single-chain variable fragment (scFvs) against PD-1 or PD-L1 | Promotes inflammatory response by inhibiting PD-1 or PD-L1\(^{64}\) |