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BEST PRACTICES FOR ONCOLYTIC VIRUS DESIGN, DEVELOPMENT, AND MANUFACTURING

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Oncolytic viruses, which exclusively target and break down cancer cells, have the potential to revolutionize standard cancer treatment. Though researchers are continuing to pursue the optimization of these viruses and their manufacture through a variety of viral engineering strategies, their continued progress hinges on meeting a number of goals linked to safety, efficacy, and commercial scale-up. These include high virus yields, highly reproducible key quality attributes, genetic stability, and formulation and product stability goals.

The relatively short history of commercial success linked to oncolytic viral therapies – the first oncolytic to receive FDA approval was in 2015 – has meant a concomitant dearth of expertise in the field of oncolytic viral design. As biopharmaceutical manufacturers begin to explore the potential of these therapies more widely, the need for contract development and manufacturing organizations (CDMOs) with experience in viral vector design, including oncolytic viral design, has become more relevant than ever.

THE PITFALLS OF ONCOLYTIC VIRUS DESIGN AND HOW TO AVOID THEM

Oncolytic viruses (OVs) are particularly promising because they possess two primary modes of action: they are capable of both killing infected cancer cells and stimulating cross-primes anticancer immunity to boost the killing of uninfected cancer cells. Because of this, they offer the possibility of greatly enhancing existing cancer therapies. Current research in oncolytic virus design is focused on arming OVs with a variety of transgenes to increase their immune stimulation, modulate immune checkpoints, and provide imaging targets. By working to synergize OVs with other immune modulators or cytotoxic agents, many drug developers hope to achieve the most potent immunotherapies for cancer possible.

Though successful commercialization of OVs as immunotherapeutics is relatively recent, the science at its core has been around for more than a century, ever since scientists first observed an abatement in cancer progression in some patients with active microbial and viral infections. In the last few decades, this observation with natural pathogens has been applied to a range of engineered viruses, including HSV-1, adenovirus, poliovirus, measles, and others. The understanding behind the mechanisms of action for these viruses has improved steadily in that time, but development is still largely early stage – besides Talimogene laherparepvec (T-VEC), a recombinant HSV-1 oncolytic virus approved by FDA and EMA in 2015, only a few adenovirus-based oncolytic therapies have been approved by regulators in China and other countries. However, numerous viral oncolytic therapies, built using a wide variety of viral platforms, are currently in clinical trials, with many showing promising results.

Part of the challenge that has served to slow the development of these therapies has been in ensuring the relative safety of the viral vector. Most oncolytic viruses are conditionally or partially replicative, primarily in tumor cells, but minimizing the potential for viral replication in healthy cells is the foremost consideration for early-stage clinical trials. This heightened safety profile places increased emphasis on the earliest phases of viral vector design, from cell line selection to infection optimization. For biologics which can self-replicate, even ones that are limited to particular cell types or cycle phases, ensuring that the virus is incapable of reverting to a fully replication-competent virus is critical to facilitating a therapy's progression along the developmental pipeline.

Because non-retroviral oncolytic viruses are living systems, another variable that researchers must account for is preventing them from integrating into the host's genome. The possibility of random recombination, particularly for patients with concomitant infections with other viruses, is another factor for which researchers must test. The transgene incorporated in these viral vectors to improve the immune response must also be thoroughly studied in order to eliminate the potential for homologous sequence recombination, including intra-molecular recombination that leads to vector instability. Authorized recombinant viral vaccines expressing RNA virus antigens, which include live recombinant adenoviral vectors, are an example of the importance of emphasizing a foundational understanding of how each class of virus functions following infection. RNA viruses have evolved outside of the nuclear environment unexposed to cistron splicing, while dsDNA adenoviral vaccines expressing a recombinant RNA virus glycoprotein will transcribe the recombinant RNA virus antigen sequence inside the infected cell nucleus in the presence of the spliceosome. Therefore, unless the RNA virus transgene sequence is engineered to remove potential splice donor/acceptor sites, dsDNA viral vectors have the potential to generate unintended splice variants resulting in the production of truncated, mis-folded, or soluble glycoprotein antigen variants with unpredictable effects on antigenicity and safety. This risk is mitigated in the encapsulated mRNA class of vaccines that, similar to the native RNA virus, are directly translated outside of the nucleus. Concomitantly, the risk of mRNA splicing and sequence integration events is inherently low.

Regardless, reducing or eliminating the potential for unforeseen scenarios often comes down to fundamentals. Vector design and engineering, combined with other facets of early product development, are critical to diminishing the potential for off-target cell replication or expression following administration of the drug product to the patient. Additionally, while the choice of medium used to grow a cell culture should not impact its safety, it can affect its productivity, making media selection an important component for ensuring optimal scale-up.

PARTNERING FOR REGULATORY, ANALYTICAL, AND PROCESS-LEVEL EXPERTISE

Vetting a CDMO partner for their experience and understanding related to viral vector design comes down to securing the basics: poorly designed vectors, deficient cell lines, and inadequate regulatory and analytical protocols are the surest ways of delaying and derailing viral vector scale-up. By engaging with a manufacturing partner with a proven track record of successful viral vector design, process design, and GMP manufacturing, as well as a demonstrated understanding of the primary challenges that plague oncolytic virus design, companies can expedite and optimize the development process, paving the way for faster, more streamlined commercial acceptance.

Supporting oncolytic viral vector design requires a focus on both the evolving regulatory requirements that apply to these therapies and the increased analytical testing needed to vet their efficacy and safety. While testing viral vectors for use in vaccines and in oncolytic applications is largely aligned, there are a few key differences: usually, oncolytic viruses possess additional active recombinant components, typically immune modulators, necessitating more intensive testing. Viral vector design for both is a rigorous exercise; engaging a CDMO partner with experience in either space can go a long way toward streamlining viral vector design and process scale-up.

Relying on this experience is equally helpful in managing regulatory expectations; because only a handful of oncolytic viral therapies exist on the market today, the regulatory environment surrounding them is still relatively fluid. Having a partner with experience engaging with regulators on oncolytics and interpreting regulatory guidance for these therapies is as critical as any process development step. Because each virus utilized in viral vector applications possesses unique features, a regulatory paradigm marked by distinctive approval paths for different OVs based on the selected virus and mode of action is probable, and will likely necessitate the use of manufacturers with expertise in vector scale-up across a wide variety of live viral vectors.

At IDT Biologika, our approach to viral vector scale-up is well codified, the result of our extensive experience with both viral vaccines and oncolytic viral development. This process begins with production cell line selection and encompasses adherent and suspension culture growth, cell lysis, media and buffer optimization, viral purification, and aseptic manufacturing, including for larger viruses, such as pox viruses, which cannot undergo the same sterile filtration steps as adenovirus and other small, often non-enveloped viruses. This end-to-end development is supported by facilities and equipment tailored to scaling up different viral vectors for a range of applications, as well as a commitment to the highest levels of GMP compliance and sterility – IDT employs single-use, disposable assemblies wherever possible and maintains the necessary levels of GMP change management and cleanroom classifications to prevent any potential cross-contamination.

At our Rockville facility, IDT has supported the development of several viral vector applications in the last year alone. This work has included pox viruses, simian and human adenovirus, cytomegalovirus, and measles virus. The expertise IDT has gained from both recent and historical scale-up in the space allows us to offer clients insight into their existing production process, from project inception to downstream development, in order to fully characterize the process and determine whether it represents the optimal approach for production. It has also afforded IDT experience in finding workarounds for many of the stumbling blocks that can hinder development; as one example, we can utilize vector designs that suppress transgene expression during the manufacturing process, circumventing issues wherein a transgene may be toxic to the production host cell.

Ultimately, proper vector design is critical. Poorly designed vectors, or the use of non-optimized cell lines, can lead to genetic instability, including the potential to generate fully replication-competent viruses, as well as the generation of transgene variants or isoforms that may impact the virus' efficacy and safety. Similarly, the impact of cell line selection, culture type, culture media, supplementation, infection optimization, and early downstream harvest processes on the viral yield and purity warrant a comprehensive, multifaceted approach. IDT's experience in optimizing not just the cell line and passage range, but the media and viral harvest conditions, offers customers the latitude to pursue these underexplored, highly transformative therapies with more confidence.

ABOUT THE AUTHOR

Trevor Broadt has served as the Analytical Development and QC director for early-phase biologics development and manufacturing at IDT, with over 25 years of industrial molecular biology experience. He is the co-author of several publications and patents in the field of oncolytic viral therapy design manufacturing and analytics.

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ABOUT THE COMPANY

IDT Biologika is a global biopharmaceutical contract development and manufacturing organization that specializes in the production of innovative live viral vaccines, viral vectors for gene & immune therapeutics, oncolytic viruses, virus-like particles and other sterile liquid or lyophilized biologics to improve human health worldwide.