ABSTRACT

The aim of this review is to provide practical information on the handling, storage, and administration procedures for personalized oncolytic adenoviruses (PTAVs), which have recently entered clinical trials. As described herein, personalized oncolytic viruses refer to transcriptionally attenuated (TA) type 5 adenoviruses that are engineered to carry one or more neoantigenic transgenes derived from patient tumors.

Vials of personalized viruses should be stored at \(-60^\circ\text{C}\) without refreezing after thawing to maintain infectivity. To prevent accidental exposure and transmission, full implementation of universal precautions for preparation, administration, and handling is required. Contaminated materials that come into contact with personalized viruses should be properly disposed of in accordance with local institutional procedures. Severely immunocompromised or pregnant healthcare workers should not prepare or administer personalized viruses or directly contact injection sites. Personalized viruses are administered subcutaneously and intratumorally; however, only subcutaneous injection will be considered in this review.

The specific storage, handling, administration, and safety requirements for personalized viruses are easily managed in the context of a clinical trial following the directives from the study protocol.

Introduction

The term “oncolytic virotherapy” refers to the administration of replication-competent viruses that selectively infect, replicate, and lyse tumor cells with defects in innate immunity, interferon responsiveness and RB/E2F/p16, p53, PKR, EGFR, Ras, and Wnt signaling pathways and, in the process, elicit an endogenous immune response against them. The viruses may be “armed” with genetically engineered therapeutic transgenes or “unarmed”.

Adenoviruses (Ad), \(^1\) discovered in 1953, and associated in humans with mild cold-like symptoms, possess distinct advantages for oncolytic virotherapy.\(^2\) These advantages include the following: a lytic replication cycle, high stability, ease of manufacture at high titers, the availability of animal models such as Syrian hamsters and cotton rats supporting Ad replication, efficient genome transfer, low pathogenicity, the lack of any neoplastic or chronic disease associated with adenoviruses in immunocompetent patients, and long term accumulated clinical experience (over a decade) from multiple phase I and phase II trials demonstrating favorable safety and tolerability.\(^3,4\) Only in one isolated and well-known case of a clinical trial participant with severe ornithine transcarbamylase deficiency was an adenoviral vector directly responsible for the death of a patient.\(^5\) In contrast, the main adverse events experienced with ONYX-015, as the most extensively tested ‘un-armed’ oncolytic virus (OV), along with its successor, H101, approved and marketed in China under the name Oncorine\(^6\) for the treatment of head and neck cancers, were mild short-lived flu-like symptoms in >90% of the patients and transient grade I/II hepatic toxicities in about 20% of the patients.\(^7\)

In contrast to the side effects of chemotherapy, which tend to increase with successive doses, no cumulative toxicity was observed with ONYX-015. A similar benign adverse event profile has been observed in cancer clinical trials with other oncolytic adenoviruses. Importantly, while preexisting anti-Ad neutralizing antibodies do not attenuate oncolytic activity following intratumoral injection, their presence generally prevents vector spillover from the tumor to the liver and lungs.

As described herein, personalized viruses refer to replication-conditional oncolytic adenovirus type 5 owned and manufactured by EpicentRx Inc. that selectively infect cancer cells and express multiple patient-specific immunotherapeutic proteins based on tumor DNA sequencing data. The rationale for the use of PTAVs (Personalized Transcriptionally Attenuated Oncolytic Adenoviruses) is that every tumor is different, having evolved complex and novel adaptations,\(^8\) which requires personalized rather than conventional ‘one-size-fits-all’ treatment. Viral modifications to tune or enhance cancer cell specificity and intratumoral spread as well as to overcome localized malignancy-mediated immunosuppression include:
(1) targeted 50 base pair deletion of two transcription factor-binding sites for Pea3 and one transcription factor-binding site for E2F1 in the E1a promoter region, which compromises productive infection in normal cells.\(^9\)

(2) insertion of a transgene in the anti-apoptotic E1b-19k region. In lieu of a generic immunostimulatory transgene like GM-CSF, the transgene in personalized viruses code for mutated peptides identified on whole-genome sequencing of individual patient tumors.

As a result, the transcriptionally attenuated virus only fully replicates in cancer cells and the immunogenic antigens/epitopes that are released by the virus will be taken up by dendritic cells and stimulate CD8\(^+\) T cell responses, making this a patient-specific mutation-based anticancer vaccine.

Hence, direct intralesional injection of PTAVs is feasible to induce local and potentially abscopal tumor regression. However, an abscopal antitumor effect is dependent on several steps, namely, the uptake of antigen by undifferentiated dendritic cells and presentation of processed antigen by mature dendritic cells to cytotoxic and helper lymphocytes, respectively, in the draining lymph nodes. Since a systemic immune response depends on the presentation of cancer neoepitopes to the residing lymphocytes in lymph nodes, the PTAV may also be injected subcutaneously over accessible, nondiseased, superficial inguinal lymph nodes where viral replication is abortive (viral gene expression is attenuated to a level too low to allow replication of enough progeny virions to sustain an infection) but the neoantigenic transgenes can still be expressed. Indeed, first-generation adenoviral vectors intended for gene therapy with E1 completely deleted and without any viral replication could only persist briefly in-vivo before low-level “leaky” expression of viral genes caused the transduced cells to be cleared by the immune system. Preclinical results from an immunocompetent mouse model suggest that lymphatic uptake of the PTAVs after subcutaneous administration reprograms the local lymph node environment to promote inflammation and neoantigen specific systemic expansion of cytotoxic T cells.

The personalization of PTAVs, custom-made to vaccinate specifically against the cancer-associated epitopes of each patient that is treated, sets them apart from all other oncolytic viruses under development by giving a rationale for subcutaneous rather than strictly requiring intralesional injection. Oncolytic viruses like talimogene laherparepvec do not have a mechanism to induce an immune response against cancer-associated epitopes unless they are injected intralesionally and induce inflammation within a tumor. In contrast, PTAVs carry cancer-associated epitopes as part of the viral genome, allowing them to be expressed and recognized as viral associated antigens even if the virus is injected into normal tissue (Figure 1).

Since personalized viruses have never been dosed in the clinic before, this review was written with clinical sites in mind to introduce PTAVs and address practical considerations regarding their handling, storage, and subcutaneous administration procedures as well as the potential for cytokine release syndrome and pseudoprogression.

**Storage**

Experimental, personalized transcriptionally attenuated viruses (PTAVs) are only supplied to approved study sites that are

![Figure 1](image-url). Subcutaneous injection of the PTAV is thought to mediate anti-tumor immunization through maturation of the dendritic cells (DCs). DCs present the expressed neoantigens from the virus to tumor-reactive CD4 and CD8 T cells, resulting in abscopal T cell-mediated regression of distant tumor nodes.
Dosage and administration

After thawing of the PTAV vial at room temperature without heating or shaking (gentle swirling is permitted), the vial should be checked immediately for particulate matter. The solution may look slightly turbid which should not be confused with particulate formation. The cap should be wiped down with 70% ethanol before withdrawal of the desired volume in a syringe. Refreezing is not permitted. Undiluted virus is stable for at least six hours at room temperature after thawing. Any unused PTAV must be disposed of in accordance with local institutional guidelines/procedures to minimize environmental or personnel exposure. Standard precautions during preparation or administration of PTAV include use of gloves, gown, mask, eye protection or face shield and covering of any exposed wounds. After the injection site near the inguinal lymph node basin is identified, skin disinfection is performed with an alcohol-saturated wipe and the alcohol is allowed to evaporate fully to prevent possible inactivation of the virus. The injection site is also marked with a large circle or an “X” from a permanent marker pen. The site is then ready for direct subcutaneous injection of the PTAV.

Since injection volumes are small, use of a 1 mL syringe is recommended. Pretreatment with topical or local anesthetic agents are also permitted but should not be mixed together with the PTAV in the same syringe. (See Figure 2)

Following injection, the site is covered with sterile gauze, and gentle pressure is applied for at least 30 seconds to stop any bleeding before swabbing the area with alcohol. Gloves should be changed and the injection site covered with a bandage for at least one week. If the bandage falls off and needs to be replaced, the patient should wear gloves. Used bandages should be placed in sealed plastic bags before disposal in the household trash. Close contacts that are pregnant, newborn or immunocompromised should avoid exposure to the injection site.

Risk of cytokine release syndrome

In thousands of patients that have received therapeutic adenoviruses, only one patient (with an underlying metabolic disorder) has died. On the basis of this prior extensive clinical experience, the risk of severe cytokine release syndrome (CRS) and toxicity in general is deemed to be low with PTAVs, especially since the neoantigens expressed by the virus are not normally present in normal tissues and, therefore, cross-reactive T cell responses are unlikely.

In case of signs and symptoms of severe CRS e.g., hypotension requiring vasopressors, neurotoxicity, mental status changes, respiratory issues such as shortness of breath etc. ICU admission and treatment with high dose steroids and tocilizumab are recommended. In case of less severe Grade 1 CRS (Grades 1 and 2) recommendations are to treat with IV fluids, Tylenol, possibly antibiotics and close careful observation. Grade 2 CRS is potentially treated with IV fluid bolus for hypotension and supplemental oxygen.

Risk of pseudoprogression

Pseudoprogression or tumor flare refers to apparent tumor progression that stabilizes or regresses over time. Radiological imaging of virally treated tumors have commonly demonstrated pseudoprogression within weeks of treatment, likely due to inflammation, followed months later by tumor regression. Immune cell infiltration is believed to underlie transient enlargement or worsening of tumors treated with
oncolytic viruses and is anticipated with PTAVs. (See Figure 3.)

**Patient case presentation and histories**

Patients that have been granted Food and Drug Administration (FDA) approval to receive treatment with PTAVs are summarized in Table 1.

**Discussion**

Cancer is a lethal and heterogeneous disease that is thought to arise from DNA mutations. While a one-size-fits all non-personalized treatment is most often used today with chemotherapy, so-called personalized therapy with targeted agents, is increasingly used to treat patients with signature mutations in genes such as EGFR, BRAF, HER2, MET, ALK and ROS1. These mutations are more commonly referred to as “driver mutations” because they push or drive the growth of the tumor cells, providing them with a selective advantage over their non-malignant counterparts. Driver mutations are distinguished from “passenger mutations” that, while present, do not increase the fitness of the tumor cells and, as such, are not responsible for cancer phenotype.14

The goal of personalized medicine is to stratify patients according to the presence or absence of specific treatable driver mutations (e.g. mutant EGFR or an ALK gene rearrangement) identified via high-throughput DNA sequencing, known more commonly as “next-generation” sequencing (NGS). The President’s Council of Advisors on Science and Technology defines personalized medicine as “the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease or their response to a specific treatment”.15

Hence, as a label, personalized medicine is misleading and somewhat of a misnomer because by the above definition it isn’t personalized at all; a more accurate label might be depersonalized medicine since treatment is centered on groups of patients, rather than individuals, that share well-characterized common or generic mutation types, molecular aberrations or pathway abnormalities, which are targetable with “off-the-shelf” small molecule inhibitors. Even CAR T cells, although they are manufactured for individual patients with autologous cells, are armed with the same receptor targeting the same molecule for each patient.

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**Figure 2.** Administration protocol.

**Figure 3.** Pseudoprogression is categorized as transient enlargement or worsening of tumor followed by stabilization or regression. Immune cell infiltration is believed to underlie transient enlargement or worsening of tumors treated with oncolytic viruses and is anticipated with PTAVs.
One of the problems with targeted therapies is that they are too targeted: only small numbers or subgroups of patients have shared “public” mutations that are amenable to treatment with these agents. The vast majority of mutations, which are specific or “private” to the individual patient, are currently considered “undruggable”, because there is no obvious approach or strategy to inhibit their function or take advantage of their presence; these individual mutations and alterations are collectively referred to as the mutanome or antigenome. Accordingly, the efficacy of these off-the-shelf targeted therapies tends to be limited, even initially, since particular driver mutations play more or less of a role in tumor progression depending on the individual, his or her treatment background and the relative importance of the mutanome.

If driver mutations are the most visible tip of the cancer iceberg, then underlying that is the bulk of the mostly undruggable mutated genes that comprise the mutanome and act “epistatically” to influence tumor growth and progression. The word “epistasis” is defined as gene-gene interactions. Because all genes in the tumor operate epistatically, both in a cooperative and competitive sense, and not in a vacuum, any cancer therapy, which purports to be personalized, should address the polygenic nature of the mutanome.

Predictably then, ‘personalized’ therapies targeted only to driver mutations inevitably develop pharmacologic resistance as the cancer cells dynamically adapt gene expression to the stress of treatment by a switch in driving mechanisms. Thus, a need exists for true personalized therapies that improve immunity to cancer and which are explicitly tailored to an individual patient.

Personalized transcriptionally attenuated viruses describe a multi-step ‘epistatic’ peptide vaccination platform method that is adaptive to the course of disease and bespoke to individual patients (one treatment per and for one patient i.e., N-of-1 rather than 1-of-N), involving

1. Whole genome sequencing of patient tumors with next generation sequencing (NGS)
2. Identification from this sequence of neoantigens that are expected to provoke an immune response but which are currently ‘undruggable’ (i.e., no pharmacologic inhibitors exist) and/or expressed at such low levels that the T cell response to them is weak
3. Manufacture of an oncolytic virus with insertion of one or more of these neoantigens
4. Subcutaneous injection of the virus
5. Repeat of steps 1–5 when and if the tumors become resistant to these particular peptides either because of tumor escape or selection of variant variants with loss of target peptide i.e., the tumor is re-sequenced, new antigenic genes are identified, a new oncolytic virus with insertion of these genes is manufactured by EpicentRx, Inc. and the patient’s tumors are re-infected with the virus. In theory, this patient-specific method of treatment is infinitely repeatable: as the tumor adapts so does the treatment until the tumor is cured or changed to a chronic disease that is manageable over the long-term. Additive or synergistic effects with approved targeted therapies or cancer chemotherapies are anticipated.

In contrast to targeted therapies which can only hit druggable, activated oncogenes, PTAVs can target undruggable genes and loss-of-function mutations in tumor suppressors. In contrast to CAR T cells which must target a surface protein have faced challenges with solid tumors because of a lack of cancer-specific targets, any proteins including completely intracellular mutated transcription factors can be targeted with PTAVs as they are processed into peptides and displayed on class I MHCs.

To date, PTAVs are only available through compassionate use protocols; however, submission to the FDA for an Investigational New Drug application to treat multiple patients in Phase 1 and Phase 2 clinical trials is in progress. The aim of this manuscript is to provide an overview of PTAVs and some of practical considerations and best practices throughout the chain of storage, handling and subcutaneous administration, information, which will hopefully serve as the starting point for dialogue with clinical sites that are interested in and/or plan to dose these agents. While special operational attention is required in compliance with the protocol and Good Clinical Practice, the requirements for safe storage, handling, transport and administration should not deter from its use in the clinic. With sufficient clinical data, it may be possible to extrapolate from this personalized treatment paradigm to subsets of the population or even the population at large.

**Table 1. Patient summaries.**

| Patient 1                                                                 |
|---------------------------------------------------------------------------|
| 48 year old white male with KRAS wild-type metastatic sigmoid colon cancer |
| Previous treatments included FOLFOXIRI plus bevacizumab, cetuximab,       |
| Yttrium-90 and atezolizumab                                               |
| Based on next generation sequencing (NGS), a 37-mer of patient specific  |
| neoantigens were inserted into the virus                                  |

| Patient 2                                                                 |
|---------------------------------------------------------------------------|
| 30 year old white male with high-grade neuroendocrine cancer of the prostate |
| Previous treatments included cisplatin / etoposide, the experimental agent |
| RRx-001, ipilimumab and nivolumab and a retil of platinum etoposide       |
| Based on neoantigen identification from NGS, the adenomatous polyposis    |
| coli (APC) tumor suppressor gene was inserted into the virus              |

| Patient 3                                                                 |
|---------------------------------------------------------------------------|
| 64 year old white male with metastatic colorectal cancer                   |
| Previous treatments included FOLFRIRI/Tirbitux, Xelox, Xeloda/Avastin,    |
| Regorafenib, Erlotinib for brain metastases, and FOLFRIRI/Avastin         |
| Based on neoantigen identification from NGS, the adenomatous polyposis    |
| coli (APC) tumor suppressor gene was inserted into the virus              |

**ORCID**

Elisa Insel [http://orcid.org/0000-0003-1358-0799](http://orcid.org/0000-0003-1358-0799)
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