# Viral Vector-Based Melanoma Gene Therapy

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## Definition

Gene therapy applications of oncolytic viruses represent an attractive alternative for cancer treatment. A broad range of oncolytic viruses, including adenoviruses, adeno-associated viruses, alphaviruses, herpes simplex viruses, retroviruses, lentiviruses, rhabdoviruses, reoviruses, measles virus, Newcastle disease virus, picornaviruses and poxviruses, have been used in diverse preclinical and clinical studies for the treatment of various diseases, including colon, head-and-neck, prostate and breast cancer as well as squamous cell carcinoma and glioma. The majority of studies have focused on immunotherapy and several drugs based on viral vectors have been approved. However, gene therapy for malignant melanoma based on viral vectors has not been utilized to its full potential yet.

## 1. Introduction

Melanoma, or malignant melanoma, represents a cancer type that develops in melanocytes known as pigment-containing cells [1]. Since the beginning of the 21st century, melanoma has remained one of the most fatal malignancies. Most patients, when diagnosed early, are treated by local surgical excision following sentinel lymph node biopsy [1]. The incidence varies by country, skin phenotype and sun exposure. It mostly affects young and middle-aged female populations (below the age of 50 years), but more males are affected from the age of 55 onwards [1]. In men the incidence of melanoma is three times higher than in women by the age of 75. Ultraviolet (UV) light is known to be the main cause of malignant melanoma. A history of sunburn in childhood or adolescence has been suggested to be directly associated with the development of melanoma. Other risk factors include the number of melanocytic nevi, family history and genetic background. It has also been confirmed that patients with a previous history of melanoma are more prone to develop multiple primary melanomas [1]. In contrast, other environmental factors like alcohol or tobacco consumption have not been associated with melanoma development [2].

Skin melanoma has generally been classified according to the origin of the sun exposure, the degree of cumulative UV exposure, age at the time of diagnosis, types of oncogenic drivers and the mutational load [2]. It is known that B-Raf proto-oncogene (BRAF), neurofibromin 1 (NF1) and NRAS mutations, together with a high mutational load related to UV exposure, are the main genetic drivers [3]. Cases of periodic sun exposure are usually associated with $BRAF^{V600E}$ and a lower mutational load [2]. It is important to mention that each melanoma subtype may evolve from different precursor lesions, which can involve different gene mutations as well as different transformational stages [3].

Current medical treatments include various methods. Most patients with recently diagnosed melanoma have early-stage disease and can be treated by surgical excision, which is curative in the majority of cases. Some treatment methods involve lymph node biopsies in addition to standard surgical excision. Unfortunately, 10% of all melanoma cases are diagnosed at an advanced/late stage and are already metastatic, including visceral and brain metastases [2][3][4][5]. These patients have a poor prognosis and the probability of treatment success is lower. For patients with advanced stage disease, revolutionary therapy agents including RAF (Rapidly Accelerated Fibrosarcoma) and MEK (Mitogen-activated Protein Kinase) kinase inhibitors as well as immune checkpoint inhibitors like anti-CTLA4 and anti-PD1 have been approved, in 2011 and 2016, respectively [5][6][7][8]. Anti-PD1 and anti-CTLA4 antibodies (nivolumab, pembrolizumab and ipilimumab) as well as BRAF and MEK inhibitors (vemurafenib and trametinib) have shown promising results in clinical trials [9][10][11][12][13][14][15][16]. Today, the presence of the $BRAF^{V600E}$ mutation is verified in clinical settings, since it determines the correct treatment strategy. Mutations in

the NRAS, NF1, CKIT, CDKN2A and PTEN genes have not been included in clinical practice yet.

Immunotherapy and kinase inhibitors are known as backbones for second-line systemic chemotherapy [17]. In the past, chemotherapy represented the treatment option for advanced melanoma. Although attempts to improve patient responses by combination therapy failed, it is still used for palliative treatment of progressed melanomas [18]. Dacarbazine, an alkylating agent, was approved by the FDA in 1974 for standard chemotherapy treatment of metastatic melanoma [19]. Despite moderate results, dacarbazine has been used as the sole standard of care, recently (in clinical trials) in combination with other chemotherapies and immunotherapies (ClinicalTrials.gov) [20]. Temozolomide (TMZ), an active metabolite of dacarbazine, has been applied in advanced melanoma [20].

Electrochemotherapy (ECT) combines two cytotoxic drugs (cisplatin and bleomycin) with high-intensity electric pulses, which enhances the delivery of the drug into cells [21][22]. This approach has been used for the treatment of cutaneous and subcutaneous melanoma nodules [23]. The overall response was 85%, and no major negative adverse events were reported [21]. Photodynamic therapy (PDT) is a light-based therapy. It represents a promising adjuvant treatment and can be used as a palliative method of choice for patients with stage III/IV cutaneous metastatic melanomas [24]. PDT is considered a minimally invasive procedure that requires a photosensitizer. Absorption is superior in metabolically active tissues [24]. The method applies non-toxic compounds, which create reactive oxygen species (ROS) when combined with oxygen [25]. ROS does irreversible damage to tumor cells and tumor-associated blood vessels, and contributes to the activation of various antitumor, immune and inflammatory responses [25][26][27][28]. Although PDT can be applied to both non-malignant and malignant diseases, several reports show that PDT alone has only limited efficacy in melanoma [22]. To improve PDT results, certain protective mechanisms, like pigmentation and oxidative stress resistance, need to be overcome [29][30]. The combination of PDT and dacarbazine chemotherapy displayed resistance reduction in pigmented and unpigmented metastatic melanomas [31]. On the other hand, combination of PDT and immunotherapy may increase the effect of eradication of the initial tumor and decrease in melanoma recurrence [29].

In the context of oncolytic viruses, their selective replication triggers tumor cell death and vector spread into neighboring cells, providing an interesting approach for cancer therapy [32]. Immunization studies in experimental animal models have employed a wide range of viral vectors based on adenoviruses, alphaviruses, herpes simplex viruses, coxsackie viruses and vaccinia viruses, targeting cancer types like glioblastoma, colon, cervix, and lung cancer as well as melanoma [33][34]. The first virus-based melanoma drug was approved by the FDA in 2015 [35].

## 2. Viral Vectors for Melanoma Treatment

As previously described, a broad range of oncolytic viruses have been evaluated for cancer gene therapy [36]. The specific targeting and killing of tumor cells and the simultaneous stimulation of the immune system have made oncolytic viruses attractive delivery vehicles [37][38][39]. This dual action promotes tumor regression as well as the induction of immune responses through innate and adaptive components. On the other hand, naturally occurring, ubiquitous, non-enveloped dsRNA viruses have shown generally mild infection in humans, and specific replication and cytopathogenicity in transformed cells, which possess active Ras signaling pathways [40][41]. Their specificity for Ras transformed cells and their relatively non-pathogenic nature in humans make them attractive anticancer therapy candidates [40][41]. This approach may lead to the recognition and removal of systemic disease and the prevention of tumor return [42].

### 2.1. Melanoma Treatment Using Herpes Simplex Virus Type 1

The prototype drug for virotherapy is an attenuated herpes simplex virus type 1 (HSV-1), which is engineered to express the human granulocyte–macrophage colony-stimulating factor (GM–CSF) [35]. The approved drug known as talimogene laherparepvec (TVEC) has the trade name Imlygic®. TVEC showed two mechanisms of action, one being the oncolytic effect of infecting and killing tumor cells at the local

injection site, and the other being the immunotherapeutic effect through induction of local and systemic immune responses [43].

TVEC replicates in tumor cells, which results in lysis and release of soluble tumor-associated antigens and viral pathogens. Migration and maturation of dendritic cells is induced by local GM-CSF expression, leading to ingestion of dissolvable tumor antigens and apoptotic tumor cells. The dendritic cells are transported to the nearest lymph nodes, where antigens initiate a systemic immune response, specifically in CD4+ and CD8+ helper and cytotoxic T-cells. However, the response rate in metastases is lower than in injected tumors, which reflects insufficient effector T-cell expansion. The other reason could be the lack of efficacy at distant sites. To overcome this limitation, combination therapy with TVEC and immune checkpoint blockers might provide better results [35].

Generally, local lytic TVEC infection in tumor cells leads to the release of various proteins, such as interferons, chemokines, danger-associated molecular pattern (DAMP), and pathogen-associated molecular pattern (PAMP). These can provide more favorable surroundings for stimulation of anti-tumor immune responses [44]. Cancer cell lysis discharges tumor-associated neoantigens for processing by dendritic cells, which are activated by TVEC-encoded GM–CSF. This could lead to stimulation of anti-tumor CD8+ T-cell responses against unrecognized antigens and this effect has been clinically demonstrated [44].

### 2.2. Melanoma Treatment Using Retroviruses/Lentiviruses

Retroviruses and lentiviruses are ssRNA, which can provide long-term transgene expression by integration into the host genome. They have frequently been used as gene therapy vectors for indications such as glioma [45][46], and breast [47], gastric [48], liver [49], pancreatic [50], and hematologic [51] cancers. One limitation of using retroviruses such as Moloney murine leukemia virus (MoMLV) for gene therapy is the requirement of cell division for transduction and integration [52]. In contrast, lentiviruses are capable of transduction of both dividing and non-dividing cells.

#### 2.2.1. Preclinical Studies with Retroviruses/Lentiviruses

Although retroviruses have demonstrated potential for treating chronic diseases such as severe combined immunodeficiency (SCID) in children [53], fewer studies have been conducted for cancer. For instance, recombinant retrovirus vectors expressing GM–CSF and IL-4 showed high-level expression in cultured primary glioma cells, which lasted for 14 days and could therefore present an attractive approach for immunotherapy [45]. However, in recent years, lentiviral vectors have replaced conventional retroviruses in gene therapy. For instance, a lentivirus carrying the EGFP reporter gene provided long-term expression in DU145 and PC3 human prostate cell lines and in vivo in pre-established and orthotopic tumors [54]. In the context of melanoma, a lentiviral vector expressing the VP22-CD/5-FC suicide gene system demonstrated superior antitumor activity in a murine uveal melanoma model [55]. In another study, a lentivirus vector expressing RNAi sequences targeting the MAT2B gene, the regulatory subunit of methionine adenosyltransferase resulted in suppressed growth, colony formation and induced apoptosis in A375 and Mel-RM malignant melanoma cell lines, and affected tumor growth in a xenograft model in vivo [56]. Moreover, antisense non-coding mitochondrial RNA (ASncmtRNAs) was downregulated by a lentivirus vector expressing short hairpin RNA (shRNA), which induced apoptosis in murine B16F10 and human A375 melanoma cell lines, significantly reduced B16F10 tumor growth in vivo, and reduced the number of lung metastases in a tail vein assay [57].

#### 2.2.2. Clinical Trials of Retroviruses/Lentiviruses for Melanoma Treatment

Related to lentivirus-based clinical trials, 30 children and adults with relapsed acute lymphoblastic leukemia (ALL) were treated with a lentiviral vector-based chimeric antigen receptor T (CAR-T), targeting CD19 (CTL019), which resulted in sustained remission with a 6-month event-free survival rate of 67% and an overall survival rate of 78% [58]. The treatment of relapsed and refractory ALL was efficient, with a

high remission rate lasting for up to 24 months. In preparation for lentivirus-based clinical trials, monocyte-derived conventional dendritic cells (ConvDCs) were transduced using a tricistronic lentivirus vector, expressing GM–CSF, IL-4 and the melanoma antigen tyrosine-related protein 2 (TRP2), to overcome the difficulties in manufacturing and potency of ConvDCs [59]. The feasibility of this approach was demonstrated with monocytes from five advanced melanoma patients indicating that a simpler GMP-compliant method for manufacturing individualized DC vaccines with a higher specificity against melanoma is possible. In another approach, to improve ex vivo manufacturing of engineered T cells, isolated human CD8+ T cells from healthy donors were transduced with a lentivirus vector expressing the gp100-specific tumor antigen-specific T cell receptor (TCR) in the presence of a novel chemical lentiviral transduction enhancer (Lentiboost) [60]. It was demonstrated that antigen-specific secretion of tumor necrosis factor (TNF) and interferon-γ (IFN-γ) occurred in the transduced cells and significant cytotoxicity was detected in the antigen-positive tumor cells, showing the potential of lentivirus-based cancer immunotherapy.

The success of CAR-T based lentivirus therapy for hematological cancers such as ALL has also triggered treatment of solid tumors [61]. However, with tumors, the transition might be limited by therapeutic barriers such as CAR-T cell expansion, persistence, trafficking, and fate. In the context of melanoma, the first results from CAR-T cell therapy could not reproduce the findings from the treatment of hematological diseases [62]. Issues to be addressed include the lack of migration of CAR-T cells from blood vessels to the tumor site, as well as the immunosuppressive tumor microenvironment within solid tumors, before this technology can be successfully applied for melanoma treatment.

### 2.3. Melanoma Treatment Using Reoviruses

The nonenveloped dsRNA Reovirus Serotype 3-Dearing Strain known as Reolysin has been shown to replicate in specifically transformed cells possessing an activated RAS signaling pathway, which makes it a potential candidate for anticancer therapy [63][64][65]. The inhibition of dsRNA-activated protein kinase (PKR) in Ras-activated cells inhibits autophosphorylation of PKR, permitting viral translation and oncolysis in tumor cells [66][43]. As Ras pathway activation occurs in approximately 60% of metastatic melanoma patients, it provides a great opportunity for Reolysin testing in malignancy treatment [67][68].

#### 2.3.1. Preclinical Studies with Reoviruses

It has been observed that some tumor cells and spontaneously transformed cell lines show favorable sensitivity toward reoviruses [69]. Other studies have revealed that intratumoral injection of reoviruses leads to regression of v-erbB-transformed NIH 3T3 or human U87 glioblastoma tumors in 80% of SCID mice. Moreover, multiple reovirus injections resulted in total tumor regression in 65% of immune-competent C3H mice [70]. The oncolytic capacity of reoviruses allows their application as single drugs targeting various types of cancers. It was confirmed that reoviruses can kill six human breast cancer cell lines (SK-BR-3, KPL4, MDA-MB-453, CRL1500, MCFT and MDA-MB-231) expressing HER-2, but not the breast cancer cell line Hs578Bst, which did not show HER-2 expression [71]. Favorable anticancer effects have also been demonstrated in immunocompetent mouse models in the presence of cyclosporine A or anti-CD4/anti-SD8 antibodies as immunosuppressant agents [72]. This shows the potential for reoviruses to be implemented for the treatment of a wide range of cancer types.

#### 2.3.2. Clinical Trials of Reoviruses in Melanoma Treatment

Reovirus-based monotherapy has been conducted for solid tumors including soft-tissue sarcomas, melanoma, breast cancer and head and neck cancer [73]. One study included 18 patients and was mainly designed to verify the safety and tolerability of reovirus intralesional administration [74]. Monitoring was done over a period of six weeks. The toxic effects were measured according to the criteria of the National Cancer Institute Clinical Trials Group. The tumor responses were measured using the Response Evaluation Criteria in Solid Tumors. After the trial period of six weeks, one patient showed complete response (CR), two demonstrated partial responses (PRs), four patients had stable disease (SD) and ten showed

progressive disease (PD) [74]. However, the results indicated that intralesional reovirus monotherapy was safe, well tolerated and did not reach dose-limiting toxicity. Local administration of reovirus in phase I/II malignant melanoma studies was as well tolerated as monotherapy [75][76]. Intravenous reovirus monotherapy was applied in a malignant melanoma phase II trial in 21 patients receiving a $3 \times 10^{10}$ 50% tissue-culture infective dose (TCID50) once every 60 min on days 1–5 every four weeks [77]. Clinical benefits as CR or PR were monitored for eight weeks. One patient demonstrated extensive tumor necrosis (75%–90%) in two metastatic lesions after two treatment cycles, whereas no other patient met the criteria for CR or PR [78]. Moreover, reovirus was detected in two out of 13 biopsies containing melanoma metastases. The findings from the phase II trial further support the positive clinical outcome obtained from phase I studies [79][80]. Furthermore, two out of 13 patients showed productive reoviral replication in melanoma metastases. Unfortunately, the trial could not progress as initially planned, as the clinical objective of having two or more patients reaching CR or PR was not achieved. According to these results, the phase II trial did not support the application of reovirus as a monotherapy for metastatic melanoma, but rather as part of a combination therapy with other therapeutic or chemotherapeutic agents [74].

### 2.4. Current Stage of Melanoma Treatment Using Coxsackievirus CVA21

CVA21, a member of the Picornaviridae family, is a nonenveloped ssRNA enterovirus enclosed in an icosahedral capsid. Two major subgroups, A and B, have been characterized in murine models [81]. Subgroup A contains 23 serotypes, with their main impact on skeletal muscles, while subgroup B contains six serotypes affecting a broad range of tissue types [79]. Their clinical significance in humans reflects their responsibility for mild upper respiratory tract infections spread by aerosol transmission [82]. The oncolytic CVA21 is commercially available as CAVATAK™ based on the wild-type Kuykendall strain [83]. Modelling of the attachment mechanisms and cell internalization has indicated that other group A serotypes may have similar oncolytic potential [84]. The CVA21 infection is characterized by attachment to the intracellular adhesion molecule-1 (ICAM-1), the primary receptor for attachment, and to the decay-accelerating factor (DAF), the secondary receptor for attachment [85]. ICAM-1 is a viral receptor common for the Picornaviridae family. Although DAF is expressed on almost all cells, its primary role is regulation of complement responses [85]. Since the attachment of CVA21 to DAF is not sufficient for host cell infection, DAF is considered to act as a membrane receptor, which accumulates the virus at the cell surface and optimizes viral entry via ICAM-1 [86]. The discovery of CVA21 and its lysis of cancer cells was mainly achieved through research conducted on ICAM-1 and DAF receptors, and comparisons between various cancer cell lines and non-malignant tissues. It has also been shown for melanoma as well as multiple myeloma, malignant glioma, breast, colon, endometrial and pancreatic cancer cell lines [87][88].

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**Keywords**

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