

Current status of gene therapy in melanoma treatment

YONGLU WANG^{1,2,*}; WEI YOU¹; XUEMING LI^{3,4,*}

¹ College of Pharmacy, Nanjing Tech University, Nanjing, China

² College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Canada

³ Nanjing Bestform Pharmaceutical Technology Co., Ltd., Nanjing, China

⁴ Postgraduate Department, Nanjing Tech University, Nanjing, China

Key words: Gene therapy, Melanoma, Viral delivery system, Nanoparticles

Abstract: Melanoma is the deadliest type of skin cancer and which has a high ability of metastasis. Surgery is an effective method to treat I or II stage melanoma patients. However, there are few treatment options for metastatic melanoma. Gene therapy is one of the attractive options and is considered as the future direction for treating melanoma. This review mainly discusses the properties and challenges of the various gene therapies in melanoma, especially the delivery systems and gene targeting.

Introduction

Melanoma is the fatal form of skin tumor which develops from melanocytes (Kee and McArthur, 2017). There was a total of about 3.1 million melanoma patients and 59800 deaths in 2015 around the world (Badea, 2017; Menezes *et al.*, 2018). Melanomas are mostly populated with Caucasian people (Vos *et al.*, 2017). Among the world, there are the highest rates of melanoma in Australia and New Zealand (Stewart and Wild, 2014). Northern Europe and North America also have quite high rates, while there are comparably low rates in Asia and Africa (Stewart and Wild, 2014; Vos *et al.*, 2017). The growing incidence rate of melanoma is also the fastest in UAS among all cancers, which increased more than 60% in two decades (Badea, 2017; Menezes *et al.*, 2018; Smith *et al.*, 2017).

Melanoma has a high ability of metastasis and develops multiple drug resistance easily, which is the main reason for the difficulty to be treated in the clinic (Badea, 2017). Current treatment includes surgery, chemotherapy, radiation therapy, immunotherapy, and molecular targeted therapy (Reghupaty and Sarkar, 2019). Surgical resection is the preferred treatment for patients in stages I or II (Menezes *et al.*, 2018).

However, surgical techniques are rarely effective for metastatic melanoma. Melanoma can metastasize lymph nodes, lungs, liver, brain, and bones, which pose a high risk for patients and are often fatal. Thus, novel treatment strategies for melanoma are urgently needed to be developed. DNA damage that causes mutations in skin cells is the main reason that prompts proliferation and the formation of

malignant tumors. Gene therapies are considered to be a radical treatment for melanoma (Schadendorf *et al.*, 2018).

Gene therapy is the most attractive area in fundamental research and clinical treatment studies. This mainly delivers therapeutic genes into an individual's cells to treat diseases. For example, to silence the expression of the pathogenic genes targeting RNA (gene knock-out), to add a protective or beneficial factor, or to modify a mutant gene.

Since 2016, FDA has approved 17 cellular and gene therapies to be marketed. Most of the products belong to *ex vivo* cancer gene therapies. Few belong to *in vivo* gene therapies. For example, gene therapies such as Novartis' Kymriah for Chimeric antigen receptor T cell therapy; Spark Therapeutics' Luxterna for RPE65 mutation-induced blindness; Axicabtagen ciloleucel for Non-Hodgkins lymphoma; Novartis' Zolgensma for spinal muscular atrophy; Alnylam's Patisiran for polyneuropathy of hereditary transthyretin-mediated (hATTR) amyloidosis in adults (Reghupaty and Sarkar, 2019). There are about two thousand clinical trials for cancer treatment that are conducting based on the data from clinicaltrials.gov, and more than seventy percent of the research is in phase I. In the past thirty years, research of gene therapies mainly focused on two core questions that needed to be solved. The first one is which delivery system should be chosen. The second one is how to screen the target genes. In this review, properties, challenges, and synopsis of the various gene therapies are provided for the treatment of melanoma, especially the delivery systems and gene targets.

Strategies of Gene Therapy

Based on the gene transfer process, gene therapies are divided into *ex vivo* gene therapy and *in vivo* gene therapy. In *ex vivo*

*Address correspondence to: Yonglu Wang, yonglu_wang777@163.com; Xueming Li, xuemingli@njtech.edu.cn

Received: 04 November 2019; Accepted: 16 April 2020

TABLE 1

Data from clinicaltrials.gov as of 10.17.2019 showing representative clinical trials that use CAR-T cells to treat melanoma

Study Identifier (number)	NCT03060356	NCT02107963	NCT03893019	NCT01218867	NCT02830724
Biological Use	T cells modified with RNA anti -cMET CAR	Anti-GD2-CAR engineered T cells	MB-CART20.1	Anti-VEGFR2 CAR CD8 plus PBL	Anti-hCD70 CAR transduced PBL
Study Status	Active, not recruiting	Completed	Recruiting	Completed	Recruiting
Delivery Route	IV	IV	IV	IV	IV
Clinical Trial Phase	Early Phase I	Phase I	Early Phase I	Phase II	Phase I/II
Country	USA	USA	German	USA	USA
Estimated Enrollment	10	15	15	24	113
Research Institute	University of Pennsylvania	National Cancer Institute	University Hospital of Cologne	National Cancer Institute	National Cancer Institute

gene therapies, cells from patients are cultured and modified in the laboratory, which enhances their ability to kill tumor cells. Then the cells are delivered back to the patient. *Ex vivo* gene is also well known as cell immunity therapy. Chimeric Antigen Receptor T (CAR-T) cells are the most representative cell immunity therapy. In CAR-T treatment, the patient's T-cells are cultured in a laboratory and are modified by viruses that code for genes that can produce different receptors. Then the modified T-cells with the receptors can target tumor cells. CAR-T therapies represent the most successful gene therapies recently. More than ten CAR-T products have been approved by the FDA since 2017, and all of them are used to treat hematological disorders. Generally, CAR-T therapies are not successful in the treatment of solid tumors, including melanoma. It is difficult for CAR-T cells to reach the tumor microenvironment and kill cancer cells (Carlino and Long, 2016). There are very few trial data about CAR-T therapies for melanoma, according to clinicaltrials.gov (Tab. 1).

In vivo gene therapy, therapeutic genes are needed to be delivered into the body using a delivery vector. Here, more than 80% percent of researches use viral vectors as the delivery method. The most common administration methods are intravenous for *ex vivo* gene therapies and intra-tumoral for *in vivo* gene therapies (Duan and Lam, 2013; Reghupaty and Sarkar, 2019). Melanoma is related to mutated or downregulated genes, which function in regulating cell pathways related to survival and death. These genes are usually ideal for gene therapy targets. In recent decades, many gene targets, such as CD20 and GD2, were proven to be effective in melanoma gene therapies. *In vivo* gene therapy represents the future directions of melanoma treatments. There are a total of 130 trial data about *in vivo* gene therapies for melanoma (Schadendorf et al., 2018). The types of gene therapies and some targets used in gene therapies are discussed below (Fig. 1).

Ex vivo Gene Therapies

The targets of *ex vivo* gene therapies for melanoma include introducing immune response modulators, modified

dendritic cells, or T-cells. The targets or biological used in CAR-T for melanoma are RNA transduced anti-cMET CAR T cells, anti-GD2-CAR, and CD20 CAR transduced T cells. MET is well-known to be a major oncogene of many tumors. cMET receptor tyrosine kinase performs key roles in cell proliferation, survival, motility, and angiogenesis. Acquired cMET amplifications are the main resistance mechanism to therapeutic drugs such as osimertinib (Collie et al., 2019; Zhou et al., 2019). A phase I study would evaluate the safety and efficacy of CAR-T cells, which express MET in patients with advanced melanoma (NCT03060356).

GD2 is another well-known cancer antigen in osteosarcomas and neuroblastoma, which is also highly

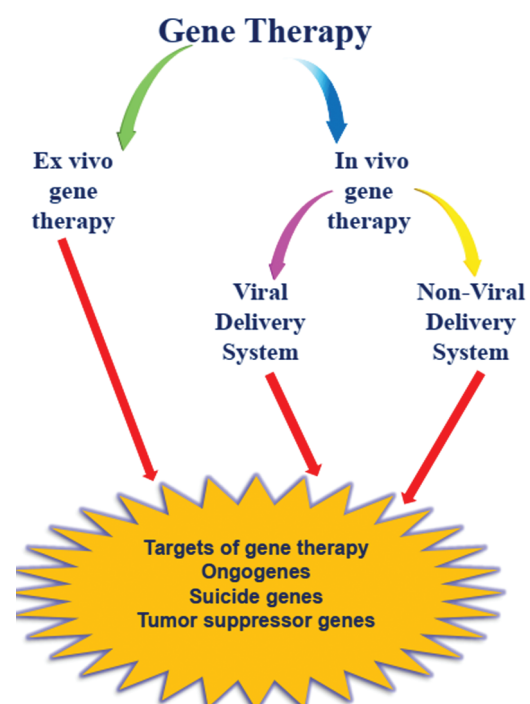


FIGURE 1. An overview of gene therapies in melanoma.

expressed in melanoma. GD2 can express in normal tissues but is restricted to skin melanocytes and sensory nerve neurons. Anti-GD2 monoclonal antibodies are suitable for the gene target therapy of melanoma (Yu *et al.*, 2010). The first generation of T cells that express anti-GD2 CARs has been proved to be safe, which are also effective for some melanoma patients. The third generation has been tested to be more effective than the first generation. The safety of the anti-GD2 CARs system is ensured by a caspase dimerization domain in the vector, acting as a suicide switch. If the vector induces toxicity, a small molecule will cause the engineered T cells to die by activating the suicide switch (NCT02107963).

The CD20 is a well-characterized tumor antigen that is highly expressed in melanoma cells and mature B cells. CD20 is a good target for the gene therapies of melanoma and B-cell lymphoproliferative disorders (Byrd, 2019). A Phase I trial in Europe will be the first trial with CD20 CAR transduced T cells targeting melanoma.

Preclinical research has also proven a strong antitumor effect of targeting CD20⁺ cells (NCT03893019). CD27 and CD70 receptors are both important regulators of T-cell function (Bak *et al.*, 2012). In another phase I/II study, researchers modified the patient's white blood cells with anti-CD70 to treat melanoma (NCT02830724).

Vascular endothelial growth factor (VEGF) plays a key role in regulating blood vessel formation, which has been widely studied in cancer research. VEGF2 is a perfect therapeutic target for numerous antitumor treatments due to its angiogenic effect (Peach *et al.*, 2018). Host antitumor immune defense is an important antitumor mechanism in patients, which is related to Tumor-infiltrating lymphocytes (TILs). CD8 + TILs are one representative component in host immune responses (Chen *et al.*, 2019; Xu *et al.*, 2019). A phase II trial study was conducted to test the effectiveness of CD8 + TILs combining VEGF2. CD8 + TILs were taken from the patient with metastatic cancer including melanoma. Then, CD8 + TILs were cultured in the laboratory and modified with genes for the anti VEGF2 receptor, which was incorporated in a retrovirus (NCT01218867).

The Targets of Gene Therapy

The main targets used in gene therapies for melanoma include suicide genes, oncogenes, and tumor suppressor genes. Oncogenes that are usually highly expressed in cancer cells have the potential to induce tumors. Many oncogenes were discovered and identified since the 1970s. Usually, oncogenes are normal genes called proto-oncogenes, which control cell proliferation or growth. If proto-oncogenes are mutated or activated, these genes will block the apoptosis process of normal cells and induce cellular canceration. The expression proteins of oncogenes were used as targets for many gene therapies (Croce, 2008; Mayr and Bartel, 2009).

Tumor suppressor genes, or antioncogenes, are genes that usually regulate cell division and replication. Tumor suppressor genes are usually expressed at low levels in tumor cells. When an antioncogene is mutated, this will result in a reduction of its function. The loss of function for tumor suppressor genes may be even more problematic than

the activation of oncogenes in the development of different human cancer cells (Mohankumar *et al.*, 2015).

A suicide gene can cause a cell to kill itself through the expression of the transgene that will convert prodrugs to cytotoxic drugs. One advantage of suicide genes is that toxic drugs produced in one cell can easily enter other nearby tumor cells that do not receive the transgene, resulting in the complete elimination of cancer cells (Zhou *et al.*, 2015).

Melanoma is a polygenic disease that is why lots of genes have been reported as the targets of gene therapies. A combination of different gene targets has become a commonly used strategy for melanoma therapy, being more efficient than one gene target. Safe and stable expression of exogenous genes efficiently in target cells are the core issues of gene therapy that highly depend on the gene delivery system. In this review, more specific targets of gene therapies for melanoma will be elaborated later together with gene delivery systems.

Oncogenes

Silencing tumor oncogenes is one effective strategy to target melanoma in gene therapies. Recently, modulating miRNA was proven to be effective in melanoma treatment (Fattore *et al.*, 2017). Several miRNAs were found involved in melanoma occurring. For example, miR-579-3p is expressed at a low level when melanoma increases (Fattore *et al.*, 2017). miR-339-3p is one tumor-suppressive miRNA that can downregulate MCL-1 expression. miR-339-3p was shown effective in blocking melanoma invasion and preventing lung metastasis (Weber *et al.*, 2016).

Suicide genes

The herpes simplex virus thymidine kinase (HSVtk) gene is the most commonly used suicide gene in melanoma therapies. The combined use of HSVtk and the nucleoside ganciclovir (GCV) is a common strategy for treating several cancers. GCV can be converted to GCV-triphosphate, which is cytotoxic to tumor cells. The expression of catalytic proteins by HSVtk induces the conversion of GCV as the first step of the phosphorylation reaction (Reghupaty and Sarkar, 2019). Usually, retroviral vectors containing HSVtk genes are injected into melanoma tissue for several times. Then a week later, GCV will be administered for about 2 weeks. There were several reports that the antitumor effects of HSVtk genes were limited, and melanomas were still progressing in patients after long-term observation (Menezes *et al.*, 2018). A phase I study reported about the insertion of a modified GCV suicide gene into a person's melanoma cells through adenovirus RSV-TK by direct intralesional injection (NCT00005057). Such treatment might make cancer more sensitive to the antiviral agent GCV. However, poor effects are the main reason that researches about suicide gene therapies are less popular in recent years.

Tumor suppressor genes

One of the anti-oncogenes commonly used for gene therapies in melanoma is MDA-7/IL-24, which has low expression levels in melanoma cells (Pradhan *et al.*, 2019). Numerous researches have proven the effectiveness of the MDA-7/IL-24

gene in treating melanoma. MDA-7/IL-24 can induce apoptosis specifically in tumor cells, whereas it has no toxic effect on normal cells (Menezes *et al.*, 2014). MDA-7/IL-24 can cause a reduction of antiapoptotic proteins such as Bcl-2 and increase proapoptotic proteins such as Bax. There was one report that MDA-7 was delivered by a cancer terminator virus (CTV) to melanoma mice, which initiated a reduction of tumor death in melanoma mice (Sarkar *et al.* 2008). An adenovirus expressing MDA-7/IL-24 gene has been tested in melanoma patients. The results showed that the adenovirus has a strong apoptosis effect on melanomas, which was also safe for patients (Menezes *et al.*, 2014).

Gene Delivery Systems

Gene Delivery Systems are mainly divided into Viral Delivery and Non-Viral Delivery Systems. Both of the delivery systems have their own advantages and barriers in clinical practice. The main progress of the viral and the non-viral vector is presented in this review.

Viral delivery systems

Vaccinia Virus, Lentivirus, and Adeno-associated Virus are mostly used as viral vectors. Vaccinia and Adeno Virus are double-stranded DNA viruses, while Lentivirus belongs to the group of single-stranded RNA viruses. The representative clinical data from clinicaltrials.gov using viral delivery systems are shown in Tab. 2.

Adenovirus

Adenovirus is one of the most frequently used viral vectors in gene therapy. One advantage of human adenovirus is that it can infect both dividing and non-dividing cells. Adenoviruses do not integrate into the host chromosomes. The adenovirus can bind to the cell membrane and then enters the cytoplasm by endocytosis. After reaching the nucleus, the viral DNA will be replicated, and viral proteins

are produced. E1B is one kind of adenovirus protein whose function is to inactivate the tumor suppressor p53 gene and promotes the viral replication in cells. Recombinant adenoviruses are usually engineered without E1B, which cannot replicate in normal cells at all. The p53 proteins in normal cells can easily recognize the recombinant viral DNA and prevent replication of the viral genome. But the p53 gene is mutated in cancer cells, so the recombinant viral DNA can replicate and finally kill the tumor cell (Dong *et al.*, 2014).

In an attempt to increase the antitumor ability of the immune system, one study combined activator ligands with the adenovirus vector designed to express protein hIL-12 (Schurich *et al.*, 2017). Cytokine-NAg fusion proteins belong to myelin-specific tolerogens. Fusion proteins containing GM-CSF and a myelin epitope are highly effective vaccine tolerogens (Islam *et al.*, 2014). ONCOS-102 is an adenovirus engineered to express GM-CSF. A phase I trial was studying the safety and effectiveness of sequential treatment with ONCOS-102, followed by pembrolizumab in melanoma patients (NCT03003676).

In the last two decades, the publications of oncolytic viruses increased by twenty times. More and more evidence has proven the effectiveness of oncolytic viruses in the treatment of various cancers, including melanoma (Zou *et al.*, 2019). Adenovirus and herpes simplex virus (HSV) are the two most thoroughly studied oncolytic viruses. A phase IIa research (NCT03190824) will evaluate the efficacy and immunological response of OBP 301 in patients with stage III and IV metastatic melanoma. OBP 301 is an adenovirus that expresses the human telomerase reverse transcriptase gene (hTERT).

CD40L belongs to the tumor necrosis factor family that is expressed in a variety of tissues. CD40L plays a major role in immune response and is a major therapeutic target for inflammation (Antoniades *et al.*, 2009; Takada *et al.*, 2019). CD40L can bind to the CD40 receptor expressed by

TABLE 2

Representative data from clinicaltrials.gov as of 10.13.2019 showing clinical trials that use viral delivery systems

Study Identifier (number)	NCT00005057	NCT01397708	NCT03003676	NCT03190824	NCT01455259	NCT00429312
Biological Used	adenovirus RSV-TK	INXN-2001	ONCOS-102	OBP-301	AdCD40L	JX-594
Study Status	Completed	Completed	Recruiting	Active, not recruiting	Completed	Completed
Delivery Route	Intralesional injection	Intratumoral injections	Intratumoral injection	Intratumoral injection	Intratumoral injection	Intratumoral injection
Clinical Trial Phase	Phase I	Phase I/II	Phase I	Phase IIa	Phase I/II	Phase I
Country	USA	USA	USA and Norway	USA	Sweden	USA
Estimated Enrollment	27	26	24	4	30	10
Research Institute	Baylor College of Medicine; Clinical Genetherapy Branch	Ziopharm Oncology	Targovax Oy	Oncolys BioPharma Inc	Uppsala University	Jennerex Biotherapeutics

dendritic cells (DCs), resulting in the activation of tumor-specific T cell responses. A phase I/II trial (NCT01455259) will investigate the effectiveness of immunostimulatory gene therapy of adenoviral vector that carrying the human CD40L gene.

Vaccinia virus

Vaccinia virus belongs to the Poxviridae family and has been widely used in cancer research. JX-594 is one of the commonly used vaccinia viruses in gene therapies. JX-594 contains functional genes such as engineered GM-CSF and viral TK. Cancer cells usually have high cellular TK activity and EGFR signaling, which will stimulate the viral replication of JX-594. Thus JX-594 can target tumor cells and has no effect on normal cells that lack TK and EGFR signaling (Breitbach *et al.*, 2015; Kim *et al.*, 2018). Melanoma has a high expression of EGFR, which makes it a suitable target for JX-594. A phase I/II trial (NCT00429312) have been performed to prove the effectiveness of JX-594 through intratumoral injection in patients with metastatic melanoma. The results also showed that JX-594 can be tolerated by all patients.

Lentivirus

Lentivirus is a single-stranded RNA virus that belongs to the Retroviridae family. During replication, the single-stranded RNA will convert into double-stranded DNA and then integrate into the host cell genome. The transgene of lentivirus will be expressed stably in host cells. Like adeno-associated viruses, lentiviruses can infect both dividing and non-dividing cells, which is an advantage for human gene therapy (Kang *et al.*, 2018). Malignant melanoma has a high expression of gp100, which is a potential target for melanoma treatment (Bol *et al.*, 2014). An early Phase I (NCT03649529) studied the effectiveness of modified lentivirus that can kill malignant melanoma expressing gp100 peptides.

Non-viral delivery system

Although more than 90% of researches in clinical trials are part of Viral Delivery nowadays, non-viral delivery systems still have some advantages in clinical applications, such as low toxicity, high targetability, good biocompatibility, long term stability, low immunogenicity, easy preparation, and multifunctional modification. The low genetic transfection efficiency is considered as the main barrier for non-viral vectors compared to viral delivery systems that limit its clinical application. However, the FDA has approved Alnylam's Onpattro, the first siRNA drug for hereditary amyloidosis in 2018, which is also the first approved non-viral vector on the market. Onpattro used the lipid nanoparticle (NP) to deliver the therapeutic siRNA directly to the liver. It is a great encouragement for research on non-viral delivery systems (Badea, 2017; Rai *et al.*, 2019).

The main advantage of NPs is that NPs can improve the targeting ability and distribution profile of therapeutic genes. Targeting moieties can easily be combined with NPs, which increases their uptake by the tumor cells. Another advantage of NPs is that building material of the NPs can be designed to release therapeutic genes at cancer cells by especially responding to the human internal environment such as pH, certain enzymes, or oxygen pressure.

Nanoparticle technologies are well established and widely used for drug delivery. There are still some barriers to the application of NPs in gene delivery. One of the most crucial barriers for non-viral delivery systems is their effectiveness, which is mainly determined by the gene transfection efficiency of NPs. The particle size, molecular weight, surface charge, and several other factors can all affect the transfection efficiency of NPs. In order to deliver therapeutic genes into the cells efficiently, there are some common requirements for NPs. For example, the particle sizes of NPs are restricted to less than 100 nm, and usually, cationic polymers are more suitable for gene delivery because they can easily conjugate with genetic materials through electrostatic attraction. Representative nanoparticle delivery systems such as Poly (ethylene glycol), gold nanoparticle, ZnO nanoparticles, and chitosan are used in gene therapies for melanoma treatment. Other polymers such as Poly (lactic-co-glycolide) (PLGA), poly(L-lysine) (PLL) and poly (ethylenimine) (PEI) are widely used in gene therapies such as liver cancer but are rarely reported in melanoma treatment (Cui *et al.*, 2019). The main reason is related to the melanoma microenvironment in the skin, which was difficult to reach for these kinds of nanoparticles.

Poly (ethylene glycol)-poly (D, L-lactide)

Phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP) is a tumor suppressor that could be a potential target for melanoma treatment. Zhang *et al.* (2019) prepared a hybrid monomethoxy poly (ethylene glycol)-poly (D, L-lactide) NP (iDPP), which was modified by an iRGD. Plasmid LHPP was delivered by the iDPP system and was tested *in vitro* and *in vivo*. The results showed that the iDPP/LHPP nanoparticle system had comparably low toxicity and significantly blocked melanoma growth with high transfection efficiency (Zhang *et al.*, 2019).

Gold nanoparticles

Carnevale and Strouse (2018) prepared a gold NP (AuNP) and nucleic acids that were bound on the surface of NPs through a covalent bond. The cell uptake, nucleic acid release, and protein expression were investigated to evaluate the effectiveness of AuNP in A375 human melanoma cells. The results showed that the intracellular rates of DNA release from the AuNP surface were highly affected by the binding activity (Carnevale and Strouse, 2018).

Wang *et al.* (2017) prepared single-guide RNA (sgRNA) targeting Polo-like kinase-1 (Plk1), using gold nanoclusters (GNs) in A375 melanoma cells. The GNs were modified by the HIV-1-transactivator of transcription peptide. The results showed that Plk1 protein expression decreased significantly in A375 cells *in vitro*, and the melanoma progress was almost blocked on mice (Wang *et al.*, 2017).

Topical delivery of genes by plasmid may be a potential technology for melanoma gene therapy. But the permeability barrier of the *stratum corneum* in the skin is the main problem that needs to be solved for topical gene treatment. Niu *et al.* (2017) prepared gold nanoparticle (AuPT) that conjugated with one kind of cell-penetrating peptide and cationic PEI. Both the cell-penetrating peptide and cationic PEI in the AuPT system can help to deliver the plasmid

DNA into melanoma cells, which increase intracellular uptake significantly (Niu *et al.*, 2017).

ZnO and MgO nanoparticles

Polyinosinic-polycytidylic acid (poly I:C) is a potential anticancer RNA. The main problems of its clinical application are the degradative hydrolytic enzymes (DNase), which will decrease its effectiveness *in vivo*. NP delivery systems are a good strategy to avoid the degradation of poly I:C by DNase. McCall *et al.* (2017) investigated the protective effect of nanoscale ZnO and MgO in serum or tumor homogenate. The results proved that both ZnO and MgO NP could protect DNA from DNase degradation (McCall *et al.*, 2017).

Polysaccharide-based chitosan

Bazylińska and Saczko (2016) prepared one kind of multifunctional nanocapsules using oppositely charged polysaccharide-based chitosan (CHIT). Herring testes DNA and fluorescent marker (IR-780 indocyanine) were loaded in the nanocapsules. The *in vitro* results showed that the nanocapsules are suitable nanocarriers for DNA delivery, which were effective in melanoma cells (Bazylińska and Saczko, 2016).

Poly (Lactic acid)

Some synthetic polymers such as PLA are studied widely for gene therapy because of their biodegradable nature. Kurosaki *et al.* (2010) prepared one novel cationic vector to deliver pDNA using cationic complexes such as PLA and PLL. In order to increase the effectiveness and safety of gene therapy, gamma PGA were coated on the surface of the cationic complexes. The results demonstrated that the gene expression of the cationic vector was high in melanoma B16-F10 cells (Kurosaki *et al.*, 2010).

Lipoplexes

Mohammed-Saeid *et al.* (2018) developed a novel gemini surfactant lipoplex system. The gemini surfactant contains phosphate and nitrogen. In an attempt to ensure the effectiveness and safety, a cancer-targeting peptide p18-4 was modified with the lipoplexes. The p18-4 in the lipoplexes system demonstrated high efficiency in human malignant melanoma (A375) cells (Mohammed-Saeid *et al.*, 2018).

Conclusion

Gene therapies for melanoma were deeply studied in the past three decades, and most research proved that gene therapies have positive results *in vivo* and *in vitro*. However, gene therapy technologies for melanoma that have reached clinical trials are limited. There are still several problems that need to be solved in gene therapies for melanoma. One common concern is the uncontrollable gene activation and inactivation caused by the integration of delivery vectors with the host genome. Gene safety problems would be screened for long term observations, using large scale clinical trials. High immunogenicity is still the main problem for viral vectors, just as low genetic transfection efficiency for non-viral vectors. Tabs. 1 and 2 reveal that

more than 80% of clinical trials are conducted in the US for melanoma treatment. The USA provides a major contribution to basic melanoma research and clinical trials.

Funding Statement: This study was supported by the National Students' platform for innovation and entrepreneurship training program (No. 201910291095Z, China).

Conflicts of Interest: The authors have no conflicts of interest to report regarding the present study.

References

- Antoniades C, Bakogiannis C, Tousoulis D, Antonopoulos AS, Stefanadis C (2009). The CD40/CD40 ligand system: linking inflammation with atherothrombosis. *Journal of the American College of Cardiology* **54**: 669–677. DOI 10.1016/j.jacc.2009.03.076.
- Badea I (2017). New strategies in melanoma therapy: can nanoparticles overcome chemoresistance? *Nanomedicine* **12**: 1623–1626.
- Bak SP, Barnkob MS, Bai A, Higham EM, Witttrup KD, Chen J (2012). Differential requirement for CD70 and CD80/CD86 in dendritic cell-mediated activation of tumor-tolerized CD8 T cells. *Journal of Immunology* **189**: 1708–1716. DOI 10.4049/jimmunol.1201271.
- Bazylińska U, Saczko J (2016). Nanoemulsion-templated polyelectrolyte multifunctional nanocapsules for DNA entrapment and bioimaging. *Colloids and Surfaces B: Biointerfaces* **137**: 191–202. DOI 10.1016/j.colsurfb.2015.07.056.
- Bol KF, Mensink HW, Aarntzen EH, Schreiber G, Keunen JE, Coulie PG, De Klein A, Punt CJ, Paridaens D, Figdor CG (2014). Long overall survival after dendritic cell vaccination in metastatic uveal melanoma patients. *American Journal of Ophthalmology* **158**: 939–947. DOI 10.1016/j.ajo.2014.07.014.
- Breitbach CJ, Bell JC, Hwang TH, Kirn DH, Burke J (2015). The emerging therapeutic potential of the oncolytic immunotherapeutic Pexa-Vec (JX-594). *Oncolytic Virotherapy* **4**: 25. DOI 10.2147/OV.S59640.
- Byrd JC (2019). Clinical trials and observations: targeting CD20 takes the backseat in CLL. *Blood* **133**: 1003–1004. DOI 10.1182/blood-2019-01-892695.
- Carlino MS, Long GV (2016). Ipilimumab combined with nivolumab: a standard of care for the treatment of advanced melanoma? *Clinical Cancer Research* **22**: 3992–3998. DOI 10.1158/1078-0432.CCR-15-2944.
- Carnevale KJ, Strouse GF (2018). Intracellular DNA cargo release from a gold nanoparticle modulated by the nature of the surface coupling functionality. *Bioconjugate Chemistry* **29**: 3429–3440. DOI 10.1021/acs.bioconjchem.8b00575.
- Chen J, Cao Y, Markelc B, Kaeppler J, Vermeer JA, Muschel RJ (2019). Type I IFN protects cancer cells from CD8 + T cell-mediated cytotoxicity after radiation. *Journal of Clinical Investigation* **129**: 4224–4238. DOI 10.1172/JCI127458.
- Collie GW, Koh CM, O'Neill DJ, Stubbs CJ, Khurana P, Eddershaw A, Snijder A, Mauritzson F, Barlind L, Dale IL, Shaw J, Phillips C, Hennessy EJ, Cheung T, Narvaez AJ (2019). Structural and molecular insight into resistance mechanisms of first generation cMET inhibitors. *ACS Medicinal Chemistry Letters* **10**: 1322–1327. DOI 10.1021/acsmchemlett.9b00276.

- Croce CM (2008). Oncogenes and cancer. *New England Journal of Medicine* **358**: 502–511. DOI 10.1056/NEJMra072367.
- Cui Y, Li X, Zeljic K, Shan S, Qiu Z, Wang Z (2019). Effect of PEGylated magnetic PLGA-PEI nanoparticles on primary hippocampal neurons: reduced nano-neurotoxicity and enhanced transfection efficiency with magnetofection. *ACS Applied Materials & Interfaces* **11**: 38190–38204. DOI 10.1021/acsami.9b15014.
- Dong J, Li W, Dong A, Mao S, Shen L, Li S, Gong X, Wu P (2014). Gene therapy for unresectable hepatocellular carcinoma using recombinant human adenovirus type 5. *Medical Oncology* **31**: 95. DOI 10.1007/s12032-014-0095-4.
- Duan F, Lam MG (2013). Delivery approaches of gene therapy in hepatocellular carcinoma. *Anticancer Research* **33**: 4711–4718.
- Fattore L, Costantini S, Malpizzi D, Ruggiero CF, Ascierio PA, Croce CM, Mancini R, Ciliberto G (2017). MicroRNAs in melanoma development and resistance to target therapy. *Oncotarget* **8**: 22262–22278. DOI 10.18632/oncotarget.14763.
- Islam ST, Curtis AD, Taslim N, Wilkinson DS, Mannie MD (2014). GM-CSF-neuroantigen fusion proteins reverse experimental autoimmune encephalomyelitis and mediate tolerogenic activity in adjuvant-primed environments: association with inflammation-dependent, inhibitory antigen presentation. *Journal of Immunology* **193**: 2317–2329. DOI 10.4049/jimmunol.1303223.
- Kang X, Wang F, Lan X, Li X, Zheng S, Lv Z, Zhuang Y, Zhao Y, Zhou S (2018). Lentivirus-mediated shRNA targeting CNN2 inhibits hepatocarcinoma *in vitro* and *in vivo*. *International Journal of Medical Sciences* **15**: 69–76. DOI 10.7150/ijms.21113.
- Kee D, Mcarthur G (2017). Immunotherapy of melanoma. *European Journal of Surgical Oncology (EJSO)* **43**: 594–603. DOI 10.1016/j.ejso.2016.07.014.
- Kim M, Nitschké M, Sennino B, Murer P, Schriver BJ, Bell A, Subramanian A, McDonald CE, Wang J, Cha H (2018). Amplification of oncolytic vaccinia virus widespread tumor cell killing by sunitinib through multiple mechanisms. *Cancer Research* **78**: 922–937. DOI 10.1158/0008-5472.CAN-15-3308.
- Kurosaki T, Kitahara T, Kawakami S, Higuchi Y, Yamaguchi A, Nakagawa H, Kodama Y, Hamamoto T, Hashida M, Sasaki H (2010). γ -Polyglutamic acid-coated vectors for effective and safe gene therapy. *Journal of Controlled Release* **142**: 404–410. DOI 10.1016/j.jconrel.2009.11.010.
- Mayr C, Bartel DP (2009). Widespread shortening of 3'UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. *Cell* **138**: 673–684. DOI 10.1016/j.cell.2009.06.016.
- McCall J, Smith J, Marquardt K, Knight K, Bane H, Barber A, DeLong R (2017). ZnO nanoparticles protect RNA from degradation better than DNA. *Nanomaterials* **7**: 378. DOI 10.3390/nano7110378.
- Menezes ME, Bhatia S, Bhoopathi P, Das SK, Fisher PB (2014). Mda-7/IL-24: multifunctional cancer killing cytokine. *Advances in Experimental Medicine & Biology* **818**: 127–153.
- Menezes ME, Talukdar S, Wechman SL, Das SK, Emdad L, Sarkar D, Fisher PB (2018). Prospects of gene therapy to treat melanoma. *Advances in Cancer Research* **138**: 213–237.
- Mohammed-Saeid W, Soudy R, Tikoo R, Kaur K, Verrall RE, Badea I (2018). Design and evaluation of gemini surfactant-based lipoplexes modified with cell-binding peptide for targeted gene therapy in melanoma model. *Journal of Pharmacy & Pharmaceutical Sciences* **21**: 363–375. DOI 10.18433/jpps30010.
- Mohankumar KM, Currell DS, White E, Boulos N, Dapper J, Eden C, Nimmervoll B, Thiruvenkatam R, Connelly M, Kranenburg TA, Neale G, Olsen S, Wang YD, Finkelstein D, Wright K, Gupta K, Ellison DW, Thomas AO, Gilbertson RJ (2015). An *in vivo* screen identifies ependymoma oncogenes and tumor-suppressor genes. *Nature Genetics* **47**: 878–887. DOI 10.1038/ng.3323.
- Niu J, Chu Y, Huang YF, Chong YS, Jiang ZH, Mao ZW, Peng LH, Gao JQ (2017). Transdermal gene delivery by functional peptide-conjugated cationic gold nanoparticle reverses the progression and metastasis of cutaneous melanoma. *ACS Applied Materials & Interfaces* **9**: 9388–9401. DOI 10.1021/acsami.6b16378.
- Peach C, Mignone V, Arruda M, Alcobia D, Hill S, Kilpatrick L, Woolard J (2018). Molecular pharmacology of VEGF-A isoforms: binding and signalling at VEGFR2. *International Journal of Molecular Sciences* **19**: 1264. DOI 10.3390/ijms19041264.
- Pradhan AK, Bhoopathi P, Talukdar S, Das SK, Emdad L, Sarkar D, Ivanov AI, Fisher PB (2019). Mechanism of internalization of MDA-7/IL-24 protein and its cognate receptors following ligand-receptor docking. *Oncotarget* **10**: 5103–5117. DOI 10.18632/oncotarget.27150.
- Rai R, Alwani S, Badea I (2019). Polymeric nanoparticles in gene therapy: new avenues of design and optimization for delivery applications. *Polymers* **11**: 745. DOI 10.3390/polym11040745.
- Reghupaty SC, Sarkar D (2019). Current status of gene therapy in hepatocellular carcinoma. *Cancers* **11**: 1265. DOI 10.3390/cancers11091265.
- Schadendorf D, Van Akkooi AC, Berking C, Griewank KG, Gutzmer R, Hauschild A, Stang A, Roesch A, Ugurel S (2018). Melanoma. *Lancet* **392**: 971–984. DOI 10.1016/S0140-6736(18)31559-9.
- Schurich A, Raine C, Morris V, Ciurtin C (2017). The role of IL-12/23 in T cell-related chronic inflammation: implications of immunodeficiency and therapeutic blockade. *Rheumatology* **57**: 246–254. DOI 10.1093/rheumatology/kex186.
- Smith RA, Andrews KS, Brooks D, Fedewa SA, Manassaram-Baptiste D, Saslow D, Brawley OW, Wender RC (2017). Cancer screening in the United States: a review of current American Cancer Society guidelines and current issues in cancer screening. *CA: A Cancer Journal for Clinicians* **67**: 100–121. DOI 10.3322/caac.21392.
- Stewart B, Wild CP (2014). World cancer report.
- Takada YK, Yu J, Shimoda M, Takada Y (2019). Integrin binding to the trimeric interface of CD40L plays a critical role in CD40/CD40L signaling. *Journal of Immunology* **203**: 1383–1391. DOI 10.4049/jimmunol.1801630.
- Vos T, Abajobir AA, Abate KH, Abbafati C, Abbas KM, Abd-Allah F, Abdulkader RS, Abdulle AM, Abebo TA, Abera SF (2017). Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* **390**: 1211–1259. DOI 10.1016/S0140-6736(17)32154-2.
- Wang P, Zhang L, Xie Y, Wang N, Tang R, Zheng W, Jiang X (2017). Genome editing for cancer therapy: delivery of Cas9 protein/sgRNA plasmid via a gold nanocluster/lipid core-shell nanocarrier. *Advanced Science* **4**: 1700175. DOI 10.1002/advs.201700175.
- Weber CE, Luo C, Hotz-Wagenblatt A, Gardyan A, Kordaß T, Holland-Letz T, Osen W, Eichmüller SB (2016). miR-339-3p is a tumor suppressor in melanoma. *Cancer Research* **76**: 3562–3571. DOI 10.1158/0008-5472.CAN-15-2932.
- Xu X, Tan Y, Qian Y, Xue W, Wang Y, Du J, Jin L, Ding W (2019). Clinicopathologic and prognostic significance of tumor-infiltrating

- CD8 + T cells in patients with hepatocellular carcinoma. *Medicine* **98**: e13923. DOI 10.1097/MD.00000000000013923.
- Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman SG, Chen HX, Smith M, Anderson B, Villablanca JG, Matthey KK, Shimada H, Grupp SA, Seeger R, Reynolds CP, Buxton A, Reisfeld RA, Gillies SD, Cohn SL, Maris JM, Sondel PM (2010). Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *New England Journal of Medicine* **363**: 1324–1334. DOI 10.1056/NEJMoa0911123.
- Zhang Q, Xiong M, Liu J, Wang S, Du T, Kang T, Liu Y, Cheng H, Huang M, Gou M (2019). Targeted nanoparticle-mediated LHPP for melanoma treatment. *International Journal of Nanomedicine* **14**: 3455–3468. DOI 10.2147/IJN.S196374.
- Zhou X, Dotti G, Krance RA, Martinez CA, Naik S, Kamble RT, Durett AG, Dakhova O, Savoldo B, Di Stasi A, Spencer DM, Lin YF, Liu H, Grilley BJ, Gee AP, Rooney CM, Heslop HE, Brenner MK (2015). Inducible caspase-9 suicide gene controls adverse effects from alloplete T cells after haploidentical stem cell transplantation. *Blood* **125**: 4103–4113. DOI 10.1182/blood-2015-02-628354.
- Zhou Y, Song KY, Giubellino A (2019). The role of MET in melanoma and melanocytic lesions. *American Journal of Pathology* **189**: 2138–2148. DOI 10.1016/j.ajpath.2019.08.002.
- Zou Y, Luo Y, Zhang J, Xia N, Tan G, Huang C (2019). Bibliometric analysis of oncolytic virus research, 2000 to 2018. *Medicine* **98**: e16817. DOI 10.1097/MD.00000000000016817.