OXFORD JOURNAL
OF STUDENT
SCHOLARSHIP

AN OVERVIEW OF CURRENT NONVIRAL GENE THERAPIES FOR METASTATIC MELANOMA

Siddharth Koneru
sidkon2021@gmail.com

ABSTRACT

Although melanoma makes up only about 1% of all skin cancer cases, it causes the majority of skin cancer–related deaths, especially when it becomes metastatic, where five-year survival rates drop to around 35%. Common treatments such as surgery, chemotherapy, and radiation therapy often fail to control metastatic melanoma because the cancer spreads throughout the body, resists treatment, and avoids immune detection. Because of these limitations, gene-based therapies have emerged as a possible alternative by allowing direct killing of tumor cells and activation of the immune system. Early research using viral gene delivery showed that tumors could sometimes be targeted and reduced, but these approaches are limited by immune reactions, risks of unintended genetic changes, small genetic carrying capacity, and high costs. As a result, interest has increased in nonviral gene delivery systems.

This literature review examines ten nonviral approaches for delivering nucleic acids or stimulating antitumor immunity in melanoma, including lipid-based carriers, electroporation, microneedles, RNA nanoparticles, and targeted gene-silencing systems. Across the reviewed evidence, several nonviral approaches reduced tumor growth in animal models, and some locally delivered therapies produced measurable activity in patients. Canine studies of lipid-based gene delivery combined with other treatments also reported substantial improvements in disease control and survival. Electroporation and RNA nanoparticle therapies currently have the strongest human evidence, whereas most other platforms remain preclinical or limited to superficial and directly accessible tumors. Several have been tested as routes for skin vaccination rather than against melanoma tumors, and this review separates those from studies that measured tumor outcomes. Therefore, nonviral nucleic-acid delivery is best regarded as a promising component of combination melanoma treatment rather than an established replacement for systemic therapy.

INTRODUCTION

While only 1% of skin cancer cases are classified as melanoma, it causes a majority of skin cancer-related deaths [1]. Melanoma occurs when melanocytes, the pigment-producing cells found near the bottom of the skin's outer layer, undergo genetic mutation, caused primarily by ultraviolet (UV) radiation exposure, and grow uncontrollably, forming a tumor. Melanoma incidence has risen: 59,944 invasive cases were diagnosed in 2007, whereas more than 104,000 were estimated for 2025 [2]. The 5-year survival rate exceeds 99% when melanoma is detected while localized but falls to 35% when it

metastasizes to distant organs, as in stage IV disease [3]. Metastatic melanoma occurs when malignant melanocytes spread from the primary tumor and establish secondary tumors in distant organs.

The spread of tumors to multiple organs contributes to lower survival, with prognosis varying by the organs affected. The rising incidence of melanoma, together with the mortality burden of metastatic disease, warrants urgent attention. Metastatic melanoma generally has higher mortality and lower median overall survival than earlier-stage melanoma [4]. Because genetic alterations enable melanoma cells to proliferate and resist therapy, current strategies do not benefit all patients, creating a need for improved treatment methods [5].

Given the increasing mortality burden of metastatic melanoma, modern therapies are being developed and tested to combat this issue, though their effectiveness is limited by the inherent therapy resistance of metastatic melanoma tumor cells. Contemporary treatment of unresectable or metastatic melanoma is centered on immune-checkpoint inhibitors, which help the immune system recognize and attack cancer cells. For tumors with certain BRAF mutations, treatment can also include drugs that block the BRAF and MEK growth-signaling pathway, while surgery and radiation remain important in selected patients and cytotoxic chemotherapy has a more limited role [6].

Surgery is useful in removing isolated metastases due to the targeted excision procedure of surgery [7]. However, in the case of metastatic melanoma, where multiple metastases have spread throughout the body, surgery is less effective due to the isolated nature of its procedure [7]. Another common therapy is the administration of powerful cytotoxic drugs to kill cancerous cells, referred to as chemotherapy. However, chemotherapy generally produces limited and short-lived responses in metastatic melanoma, making it a less reliable treatment option [7].

Radiation therapy uses ionizing radiation to damage tumor-cell DNA and is commonly applied to selected metastatic lesions or for symptom control. Radiation therapy, while showing observable success in treating metastatic melanoma, lacks durability in controlling spread metastases, limiting its overall effectiveness [7]. Overall, the difficulty of achieving durable control across multiple metastases calls for more effective treatment strategies.

An opportunity for a more effective treatment method is present in gene therapies, which have recently been developed to introduce tumor-suppressing genes or immune-activating genes to directly kill cancer cells. Gene therapies are divided into nonviral and viral vectors. Viral vectors use a virus to encapsulate genetic material, such as DNA or RNA, and deliver it to cancer cells, which is advantageous due to the virus's inherent tendency to penetrate cells. Some of the viral vectors tested for melanoma are retrovirus vectors, reovirus vectors, and alphavirus vectors. Retroviral vectors carry single-stranded RNA that is converted into DNA inside the target cell, allowing the delivered genetic material to become part of the cell's DNA.

Early retroviral melanoma studies demonstrated that gene-modified cell therapy was feasible, but the small and varied patient groups in these early clinical trials do not support broad conclusions about treatment effectiveness. Alphavirus vectors are RNA-based platforms that have shown the ability to

deliver melanoma antigens and stimulate immune responses mainly in preclinical studies. However, these studies did not use clear clinical measures of tumor response, so they do not establish treatment effectiveness. Reoviral vectors have also been used in clinical trials against melanoma. In a phase II clinical trial, intravenous reovirus did not produce objective tumor responses, although evidence of substantial tumor damage was observed in one patient [8].

While viral vectors are becoming more popular as seen in recent retroviral clinical trials, viral vectors are inherently limited by several shortcomings. These include the risk of immune reactions, harmful biological effects, and genetic changes that could contribute to cancer [9]. Furthermore, viral vectors cannot handle a large genetic material load to deliver into the body, which coupled with high production costs, add to the burden of viral vectors [10,11]. This creates an opportunity for increased research into nonviral vectors, the alternative method of gene therapy. Nonviral gene therapy uses various techniques apart from viruses to administer materials into the body to target tumor cells.

This narrative review compares ten nonviral methods for delivering DNA or RNA: lipoplexes, lipid-based nanoparticles, electroporation, microneedles, fusion peptides, RNA nanoplexes, gene gun delivery, DNA tattooing, laser microporation, and spherical nucleic acids. BO-112 is included as an RNA-based immunotherapy, although it does not transfer a gene or silence a specific gene. Several of the remaining methods have been studied mainly as routes for skin vaccination rather than for delivering a gene into a tumor. The evidence they offer therefore differs in kind from studies that treated established melanoma tumors. Nonviral systems avoid several risks associated with viral vectors, but they can still cause inflammation, unintentionally reach healthy tissues, produce toxicity from their delivery materials, or fail to release their genetic cargo efficiently inside cells. Additionally, nonviral vectors are generally easier and less expensive to manufacture and can carry larger genetic payloads. These advantages make nonviral vectors practical gene-delivery systems for cancers such as metastatic melanoma and support additional research to build on existing studies.

LIPOPLEXES

Lipoplexes are nonviral gene delivery systems made from positively charged lipids that carry plasmid DNA, which is a small circular DNA molecule engineered to express a specific gene. This process creates complexes capable of entering cells through electrostatic forces while maximizing efficiency of packaging genetic material and maintaining molecular structure under the physiological environment of the body [12]. Lipoplexes deliver genetic therapy across a range of anatomical locations through either membrane fusion or endosomal escape, while also activating innate immune responses that increase therapeutic responses and being more cost-effective compared to current viral vector methods [12]. Lipoplexes are often used as adjuvant therapies to standard treatments, such as surgery or immune system stimulation treatments, and in these studies the combined regimens produced better local disease control than surgery alone [12–14]. These related canine studies support further testing of lipoplexes, but their combined treatments do not show how much benefit came from the lipoplexes alone [12–14].

Across three related canine studies, lipoplex-based treatment produced large improvements in local disease control and survival. However, its intratumoral administration and dependence on adjuvant therapies prevent lipoplex therapy from being established as a stand-alone treatment for distant human metastases [12–14]. Across these studies, researchers treated dogs with naturally occurring melanoma using lipoplexes carrying the herpes simplex virus thymidine kinase gene (HSV-tk). This gene therapy was given with ganciclovir and immune-stimulating cytokine treatments, and later studies also included surgery as an adjuvant therapy [12–14]. Cells receiving HSV-tk can convert ganciclovir into a toxic compound that contributes to tumor cell apoptosis, allowing cytokine therapy to further the tumor attack through immune system signaling [12]. In the 2008 study, dogs whose tumors responded to this combination had an average tumor mass reduction of 80%, and the group's median survival was more than twice that of untreated controls [12].

In the 2012 study, researchers evaluated the combination after complete surgical removal. Compared with dogs treated with surgery alone, the combined treatment resulted in better local disease control, fewer distant metastases, and longer survival [13]. The 2015 regimen built on this approach by adding canine interferon-beta (IFN-β), a protein that stimulates the immune system and has antitumor properties [14]. Compared with surgery alone, the expanded combination improved local disease control and the proportion of dogs without distant metastases [14]. Taken together, these sequential studies from the same research program demonstrate reproducible local activity in spontaneous canine melanoma, but their multicomponent regimens do not isolate the independent effect of the lipoplex platform [12–14].

Although these studies demonstrated substantial control of treated tumors, their reliance on intratumoral administration limits anatomical reach to accessible lesions and therefore reduces their applicability to disseminated metastatic disease. Additionally, lipoplexes were combined with immune-stimulating therapies in all three studies and with surgery in the later studies, meaning that these studies cannot establish how effective lipoplex therapy would be on its own [12–14]. Although these studies treated dogs with naturally occurring melanoma, clinical trials with human patients are needed to establish the safety and effectiveness of lipoplex gene therapy in human subjects with metastatic melanoma [12–14]. Taken together, these limitations suggest two priorities for future research: developing delivery systems capable of reaching noninjectable metastases and determining how much therapeutic activity the lipoplex platform retains without surgery or intensive immune stimulation [12–14].

LIPID NANOPARTICLES

Lipid nanoparticles (LNPs) are small carriers that package genetic material such as small interfering RNA (siRNA). Their lipid composition affects how they travel through the body, enter cells, and release their cargo inside cells. Small interfering RNA is often associated with LNP-based therapy because these double-stranded non-coding RNA molecules are able to suppress the expression of a specific target gene through a process called RNA interference (RNAi) [15]. Although many LNPs use ionizable lipids that are neutral in circulation and become positively charged in acidic compartments inside cells,

known as endosomes, one study used inherently positively charged LNPs designed to bind to a receptor on target cells [15].

LNPs have increasingly been used to deliver siRNA that targets oncogenes, which are genes that drive uncontrolled cell growth, in order to interrupt the molecular pathways responsible for rapid cell division. LNPs have also been used alongside chemotherapy to increase antitumor effects, making them a useful tool in nonviral gene therapy [15]. Silencing a growth-related gene is therefore a plausible melanoma strategy, but whether it improves patient outcomes remains untested in these studies [15,16].

Targeted lipid-based siRNA carriers can suppress melanoma-associated oncogenes and inhibit tumor growth in preclinical models, although transient silencing and combination-dependent durability remain major limitations. Two separate studies used LNPs to deliver siRNA targeting melanoma oncogenes in mice bearing murine melanoma tumors or human melanoma cells implanted in mice, known as human xenograft models [15,16]. In the first study, researchers administered c-Myc siRNA, which is siRNA designed to suppress the oncogene c-Myc, using LNPs formulated to bind to receptors on murine melanoma cells [15]. Three consecutive systemic administrations inhibited tumor growth, with the strongest reported effect being complete suppression of measurable tumor growth for approximately one week when the LNP therapy was combined with the chemotherapy drug paclitaxel [15]. Because the most durable inhibition occurred in the paclitaxel combination arm, the study supports potential therapeutic synergy but does not establish the durability of the siRNA formulation as monotherapy [15].

The second study formulated siRNA LNPs to target PCTAIRE1, also known as cyclin-dependent kinase 16 (CDK16), a protein involved in cell growth. Direct injection into tumors reduced PCTAIRE1 expression for up to four days, though the effect weakened toward baseline afterward [16]. Repeated treatment significantly reduced tumor volume and weight compared with the control group [16]. Combined, these studies display the functional oncogene-silencing that can be induced by LNPs in living organisms, with the tumor response directly tied to the duration of the treatment's effects [15,16].

While LNPs have shown an ability to reduce oncogene expression and tumor burden through siRNA delivery, durable control after treatment ends has not been established. Specifically, the PCTAIRE1 study found that its silencing effect started weakening within days and required repeated dosing to maintain therapeutic effects, which raises concerns about practicality in clinical settings [16]. Additionally, the strongest result in the first study came from combining siRNA LNPs with paclitaxel, making it difficult to determine how much of that result came from siRNA delivery [15]. Current formulations also need improved targeting precision to reach tumor cells while limiting exposure to healthy tissue and avoiding immune recognition [15,16]. Therefore, future work should focus on extending the duration of siRNA silencing and determining whether adding LNP-based gene therapy to established treatments provides further benefit [15,16].

ELECTROPORATION

Electroporation uses brief electrical pulses to open temporary pores in the cell membrane, the lipid layer that normally keeps large molecules out, so that plasmid DNA can enter the cell before the membrane reseals [17]. Because the pulse itself creates the opening, no viral vector or lipid carrier is needed, which avoids the immune reaction those carriers can provoke [17]. In practice the treatment is given directly into a tumor, with needle electrodes placed in the tumor tissue to generate the electrical field [18]. In the studies reviewed here, the delivered DNA encoded immune-signaling proteins rather than a gene meant to correct the tumor cell itself. Delivering immune-stimulating genes straight into the tumor is what makes electroporation useful for melanoma, but it also ties the method to tumors a clinician can physically reach with an electrode [18,19].

Among the physical delivery methods reviewed here, electroporation has the most direct clinical evidence in melanoma patients. It produces local immune activation and, in a few cases, regression of lesions that were never treated, although its use stays limited to tumors a clinician can reach. One mouse study delivered plasmid DNA encoding interleukin-12 (IL-12), an immune signal that activates tumor-killing T cells, into established B16.F10 melanoma tumors by electroporation [17]. Forty-seven percent of treated mice were tumor-free 100 days later, and most of the cured animals rejected a second tumor implanted afterward, which indicates that local treatment produced lasting immunity rather than only clearing the injected mass [17]. Because the tumors were already established before treatment, this is a treatment result and not a prevention result.

A phase I trial then applied the same approach in 24 patients, injecting IL-12 plasmid into accessible melanoma lesions and immediately applying electrical pulses to each treated site [18]. Treated lesions showed rising IL-12 levels with increasing dose, visible tumor cell death, and more immune-cell activity [18]. Two of 19 evaluable patients had complete regression of distant lesions that had never received a pulse, which suggests an effect beyond the injected tumor, though two patients is far too few to treat that effect as reliable [18]. A separate mouse study combined IL-12 plasmid with plasmid encoding interleukin-2, a signal that supports T-cell growth and survival, and reported complete responses in 71% of treated animals [19]. The two mouse studies used different protocols, so this cannot be read as evidence that the combination outperforms IL-12 alone. Together, these studies support the clinical feasibility and local immune activity of electroporation for accessible melanoma lesions, but the small uncontrolled human cohort and anatomical access constraint preclude treating it as established systemic therapy [17–19].

Electroporation delivers genes directly and efficiently, but it requires needle-electrode arrays and carefully calibrated pulse settings, which restricts treatment to tumors that can be reached physically [18]. Results also depend heavily on the operator, since variation in electrode placement, tissue contact, and pulse delivery changes how much DNA actually enters the tissue and therefore how consistent the treatment effect is [19]. Future work should therefore standardize electrode and pulse protocols and test whether the responses seen in uninjected lesions occur often enough, and last long enough, to matter clinically [18,19].

MICRONEEDLES

Microneedles are arrays of microscopic projections, roughly 25 to 900 μm tall, that are pressed onto the skin to deliver nucleic acids without a conventional injection [20]. They are commonly molded from dissolving materials such as hyaluronic acid, a naturally occurring sugar-based polymer, with siRNA loaded into the needle matrix itself [20]. Once the needles pass through the stratum corneum, the outermost layer of dead skin cells that blocks most large molecules, and into the epidermis beneath it, the matrix dissolves and releases its siRNA into the surrounding tissue [20]. Gene silencing therefore happens where the needles were applied, and without the pain of a conventional needle injection [20]. This makes the method well suited to superficial cutaneous melanoma, where the tumor sits within the short reach of the needles [21]. Delivering a repeatable dose to a defined patch of skin is the main appeal of microneedles, but the same shallow reach that keeps the treatment simple also rules out deep or internal metastases [20,21].

Two mouse studies found that dissolving microneedles delivered siRNA into superficial melanoma tumors and slowed their growth, with the effect scaling to the delivered dose, though both remain preclinical and neither addressed disease beyond the skin [20,21]. The first study targeted signal transducer and activator of transcription 3 (STAT3), a signaling protein that helps melanoma cells survive and divide [20]. Microneedles carrying siRNA against it suppressed STAT3 expression within 48 to 72 hours of a single application, which confirms that the cargo was released and taken up quickly [20]. By day 13, the higher siRNA dose had reduced mean tumor volume by roughly 80% compared with untreated controls and the lower dose by roughly 50%, so the degree of tumor suppression tracked the amount of siRNA delivered [20].

A second study added a carrier to the needle matrix, combining the peptide octaarginine with polyethylenimine to help siRNA cross the cell membrane after release, and targeted a growth-driving pathway in a human melanoma xenograft model [21]. Treatment every two days inhibited tumor growth by roughly two thirds relative to control microneedles [21]. Because the tumor model, the silenced gene, and the dosing schedule all differ from the first study, these percentages cannot be set beside each other as a head-to-head comparison. Both studies show that local gene silencing can translate into measurable tumor control in lesions the needles can reach, and the added carrier in the second suggests that formulation is one lever for improving that control [20,21]. Unlike electroporation, neither microneedle study has been tested in patients, so this evidence sits one step further from the clinic even though measured tumor shrinkage is stronger than skin-penetration data alone [17–21].

The transdermal route that makes microneedles simple also caps how deep siRNA can travel, leaving tumors in the deeper dermis or subcutaneous tissue out of reach [20]. Keeping siRNA intact inside a dissolving matrix is a separate formulation problem, because cargo that degrades before reaching the cell lowers silencing efficiency and adds variability between treatments [21]. Needle length and spacing matter for the same reason, since uneven contact spreads the dose unevenly across the treated area [20]. Future work should therefore focus on formulations that protect siRNA during dissolution and on

needle geometries that reach further into the skin [21]. A separate question is whether microneedle delivery adds anything to the systemic treatment patients already receive [21].

FUSION PEPTIDES

Fusion peptides are short engineered chains of amino acids that join segments with different jobs, so a single molecule can cross barriers that normally stop gene therapy, from the stratum corneum down to the cell membrane [22]. Entry into the cell happens through receptor-mediated endocytosis, in which the cell engulfs the peptide after it binds a surface receptor, and through direct fusion with the membrane [22]. In melanoma, these peptides are built around receptors that tumor cells overexpress, which gives them a selectivity that carriers relying on passive uptake do not have [22]. Combining that receptor targeting with the ability to cross the skin without a viral component is what makes fusion peptides a plausible route for siRNA delivery to accessible melanoma lesions [22].

How reliably this works, however, depends on how much of the target receptor a given tumor expresses, and the supporting evidence so far comes from a single preclinical study. That study tested SPACE-EGF, a carrier built from two segments with separate jobs [22]. The Skin Penetrating and Cell Entering (SPACE) segment moves the package through skin and into cells, while an epidermal growth factor (EGF) segment binds the epidermal growth factor receptor (EGFR) on the melanoma cells being targeted [22]. The carrier delivered siRNA against c-Myc, a gene whose overexpression drives uncontrolled cell growth [22].

Over 13 days in tumor-bearing mice, the carrier inhibited tumor growth by about 39% when injected under the skin and about 32% when applied topically [22]. Both routes were therefore active, but neither came close to eliminating the tumor. This supports fusion peptides as a targeted nonviral carrier that turns gene silencing into modest tumor suppression, though one mouse study cannot establish benefit in patients [22]. Compared with microneedles, the carrier crosses the skin chemically rather than by opening physical channels, so the two approaches reach the same depth limitation from opposite directions [20–22].

Because uptake depends on EGFR, tumors expressing little of it would be expected to take up less siRNA and respond less [22]. The carrier's activity therefore varies with a feature of the tumor rather than with the delivery system itself. The topical route also limits how deep the siRNA travels, which confines the approach to lesions near the skin surface rather than deep dermal or subcutaneous disease [22]. Fusion peptide complexes are also at risk for having their siRNA cargo broken down by bodily enzymes during the delivery process, which reduces the ultimate gene-silencing effectiveness of the treatment [22]. The study itself pointed toward three next steps: widening the range of receptor targets, chemically stabilizing the siRNA against enzymatic breakdown, and increasing how much of the dose reaches the tumor [22]. Each would need to be addressed before the approach could be judged clinically.

RNA NANOPARTICLES

RNA nanoparticles are nanoscale carriers built around synthetic double-stranded RNA (dsRNA), a form of RNA that cells interpret as evidence of viral infection [23]. Their purpose differs from that of the siRNA carriers discussed earlier in this review. Rather than switching off a gene that drives tumor growth, they are designed to provoke an immune response inside the tumor itself [23]. Once injected, the dsRNA is detected by cellular sensors that normally watch for viral RNA, which triggers interferon signaling and draws immune cells into the surrounding tissue [23]. The intended result is to convert a tumor that the immune system has largely ignored into one it recognizes and infiltrates, which is a central obstacle in treating advanced melanoma [24]. The clinical form of this approach is BO-112, a synthetic dsRNA packaged into nanoplex particles and tested in melanoma both on its own in animals and alongside other treatments in patients [25].

In mice bearing established melanoma tumors, injecting BO-112 directly into the tumor slowed its growth and increased the activity of natural killer cells and CD8+ T cells, the two cell types most responsible for killing tumor cells [23]. This is the clearest evidence that the nanoplex has antitumor activity on its own, without a partner treatment.

A second mouse study implanted tumors at two separate sites and irradiated only one of them, injecting BO-112 into the irradiated lesion [24]. Control of the treated tumor was strong, while growth of the untreated tumor on the opposite side was only modestly delayed. That difference matters for a disease defined by spread, because it shows that local immune activation did not translate into equivalent control at a distant site.

The approach has since been tested in patients. A phase II trial gave weekly intratumoral BO-112 together with pembrolizumab, an antibody that keeps T cells active against tumors, to 42 patients whose advanced melanoma had already progressed on anti-PD-1 treatment [25]. One quarter of the evaluable patients had an objective response and a further 40% had stable disease, which is meaningful in a group whose disease was already resistant to checkpoint blockade [25]. Because every patient received both drugs, the trial cannot separate what BO-112 contributed from what pembrolizumab contributed.

BO-112 therefore has the most advanced human evidence of any platform in this review apart from electroporation, and the two share the same constraint. Both require a lesion that a clinician can reach with a needle, so the treatment is local even when the intended effect is systemic [18,23–25]. Responses also varied considerably between patients, which suggests that baseline immune status and the composition of the tumor microenvironment shape the outcome in ways that are not yet predictable [24]. The open question is not whether BO-112 does anything on its own, since the mouse work showed local activity without a partner drug, but how much it adds to checkpoint blockade in patients. Answering that requires a trial with a comparison group receiving pembrolizumab alone [25].

GENE GUN

The gene gun, also called biolistic delivery or particle bombardment, uses a burst of pressurized helium to fire gold microparticles one to three μm across into the outer layers of the skin [26]. The particles carry DNA or mRNA on their surface, and once they reach the epidermis the genetic material comes free and is taken up by the surrounding cells [26]. Among those cells are antigen-presenting cells, which display fragments of the encoded protein to T cells and initiate an immune response [27]. Because the skin is densely populated with these cells, the gene gun is used mainly for vaccination rather than for delivering a gene into a tumor [26]. Its reach is only a few cell layers deep, which rules out direct treatment of tumors in the dermis or in internal organs [26].

The melanoma evidence for this platform comes from a single mouse study, and it tests prevention rather than treatment. Mice were vaccinated by gene gun with mRNA encoding a melanoma antigen and then challenged two weeks later with tumor cells injected to produce lung metastases [26]. Vaccinated animals developed fewer metastases than unvaccinated ones, which shows that gene-gun delivery can generate antigen-specific immunity strong enough to blunt tumor seeding. It does not show that the same immunity can shrink metastases already growing in an animal, and that distinction is the one that matters for patients with advanced disease.

The second gene-gun study cited here tested a different question in a different disease. It used a T-cell lymphoma model rather than melanoma, and vaccinated the animals before tumor challenge [27]. Co-delivering a small dose of plasmid encoding a helper antigen alongside a tumor vaccine improved outcomes compared with the tumor vaccine alone [27]. The result supports helper-antigen co-delivery as a general way to strengthen gene-gun vaccination, but it is not melanoma evidence, and the benefit came from the added antigen rather than from the delivery method itself.

Because the cited work used repeated vaccination schedules and added helper antigens, how long protection lasts from gene-gun delivery alone remains unknown [26,27]. The shallow reach of the gold particles confines delivery to the epidermis, so any effect on deeper or internal tumors has to travel through the immune cells the vaccine activates rather than through the delivered gene itself [26]. Repeated dosing to sustain that immune pressure raises practical questions about scheduling and patient tolerance that have not been studied systematically [27]. Future work should establish prime-boost schedules that extend response duration and, more importantly, test whether gene-gun vaccination against a melanoma antigen can treat established tumors rather than prevent them [26,27].

DNA TATTOO

DNA tattooing delivers plasmid DNA into the skin using a tattoo device, whose oscillating needle array punctures the same patch of skin thousands of times within a few seconds [28]. The repeated punctures spread the DNA across the dermis, the living layer of skin beneath the surface, rather than depositing it at a single point [28]. They also cause local tissue damage, and the inflammation that follows recruits immune cells to the treated area [28]. That inflammation acts as a built-in adjuvant, helping dendritic

cells in the skin take up the encoded antigen and present it to T cells [28]. Compared with a single intradermal or intramuscular injection, this distributes the plasmid across far more cells per unit area [29]. The device is commercially available and no viral carrier is involved, which makes repeated administration practical, although practicality is not the same as clinical benefit [29].

Two studies support delivery and local immune activation but not tumor control. The first applied the technique to human skin outside the body and showed that a reporter gene was expressed across the treated area, which established the delivery step without testing any tumor endpoint [28]. The second delivered a DNA vaccine encoding a fragment of MART-1, a protein recognized by T cells on melanoma cells, to nine patients with inoperable stage IIIC to IV disease [29].

The clinical results were modest. A MART-1-specific T-cell response was detectable in the skin at the tattoo site, but no corresponding increase appeared in the blood of the patients who could be evaluated, and only one patient had stable disease [29]. Every patient developed a skin reaction at the vaccination site and more than half reported pain [29]. The trial was designed to assess safety and immune response rather than tumor control, so the absence of regression is not a failed efficacy result. It does mean that the antitumor effect of this platform in patients remains untested [28,29].

The immune responses produced this way have so far been local and moderate [28]. That is consistent with a route reaching only the cells near the skin surface, without amplifying the plasmid the way a replicating vector would [28]. The skin reactions seen in every patient raise a practical concern, since sustaining an immune response would require repeated sessions and irritation may accumulate across a treatment course [29]. Generating an antigen-specific response may also not be enough on its own, because advanced melanoma actively suppresses the T cells such a vaccine produces [29]. The useful next test is therefore whether tattoo vaccination adds measurable benefit alongside checkpoint inhibition, and whether the schedule that would require is tolerable [28,29].

LASER MICROPORATION

Laser microporation applies short, low-energy laser pulses to the skin to create an array of microscopic channels through its outer layer [30]. The pulses work by photothermal ablation, converting laser energy into heat that vaporizes narrow columns of superficial tissue [31]. The skin surrounding each channel is left intact and rebuilds the barrier soon afterward, so the openings are temporary [31]. A vaccine or other cargo applied to the treated area can then pass through these channels into the living epidermis and dermis below [30]. Channel depth and density can be adjusted by changing the laser settings, which is the main technical appeal of the method [30].

Laser microporation and electroporation both use a device to improve delivery, but they open different barriers. The laser removes the stratum corneum in small patches so that material applied to the skin surface can get through, and the cargo is placed on the skin after the device has been used [30]. Electroporation opens the membranes of cells inside a tumor, and the DNA is injected into that tumor before the pulses are applied [17,18]. The practical consequence is that electroporation acts on a lesion

a clinician has already identified, while laser microporation acts on healthy skin as a route for vaccination.

The available evidence differs in the same direction. Electroporation has been tested in melanoma patients with tumor endpoints, whereas the laser work cited here measured immune responses in mice and included no melanoma tumor at all [17,18,30,31]. The two methods therefore cannot be ranked against each other on efficacy, because no comparable endpoint has been measured for laser microporation in melanoma.

The experimental support for this method is narrower than its mechanism suggests. The one cited experiment applied protein antigen, not DNA or RNA, to laser-treated mouse skin and found antigen-specific T-cell and antibody responses that varied with the depth of the channels [31]. It therefore demonstrates that the laser opens a usable route into immune-active skin, but it does not demonstrate nucleic-acid delivery, and it did not include a melanoma tumor [31]. The other laser source cited in this review is a review article rather than an independent experiment, so it describes the method without contributing separate evidence [30]. These sources together establish a delivery mechanism and preclinical immunogenicity, and nothing beyond that.

How long any response lasts after laser-assisted vaccination has not been measured, and neither has the persistence of expression when a nucleic acid rather than a protein is delivered [30,31]. Reproducibility in a clinical setting would require standardized wavelength, energy, pulse duration, and channel density, none of which the cited evidence quantifies across operators [30]. Because the available work is preclinical and uses vaccination endpoints, none of it speaks to deep or disseminated melanoma lesions [30,31]. The necessary first experiment is a melanoma tumor endpoint after laser-assisted nucleic-acid vaccination. Only once that exists can combinations with other treatments be evaluated for added benefit [30,31].

SPHERICAL NUCLEIC ACIDS

Spherical nucleic acids (SNAs) are nanoparticles with short strands of DNA or RNA packed densely around a central core, most often a gold particle between 13 and 50 nanometers across [32]. Arranging the strands radially rather than leaving them linear changes how they behave. The dense outer shell shields the nucleic acid from enzymes that would otherwise degrade it, and it allows the particle to enter cells through scavenger receptors, a route that requires neither a transfection agent nor a viral vector [32]. The same arrangement provokes less unintended immune activation than free linear nucleic acids, which matters when the goal is to silence a gene quietly rather than to stimulate an immune response [32].

The cited work tested delivery rather than treatment. Applied topically to intact skin, siRNA-carrying SNAs crossed the outer barrier and reduced expression of their target gene in the skin, without any conventional transfection agent [32]. This establishes two things at once, which is unusual for a topically applied nucleic acid. The particles reached living skin cells, and the siRNA they carried was

still functional when it arrived. What the experiment does not establish is any melanoma endpoint, because it measured gene expression in skin and did not involve a tumor [32].

That places SNAs earlier in development than most platforms in this review. The microneedle and lipid carrier studies discussed above measured tumor volume in animals carrying melanoma, whereas the SNA evidence stops at gene regulation in skin [15,16,20,21,32]. SNAs should therefore be described as a cutaneous delivery platform with demonstrated gene-silencing activity, not as a demonstrated melanoma treatment.

Topical application also sets an anatomical ceiling. Passive penetration through skin can reach superficial tissue, but it offers no route to deeper dermal or metastatic lesions [32]. Keeping the particle intact is a second requirement, since loss of surface strands or breakdown of the core before uptake would reduce silencing and introduce variability between treatments [32]. The next step is to test these particles against melanoma tumors directly, and to test whether pairing them with a method that opens the skin, such as microneedles or laser microporation, increases how much cargo reaches the tumor [30–32]. Those combinations remain proposals until they are compared experimentally.

CONCLUSION

The ten approaches reviewed represent distinct nonviral nucleic-acid therapeutics and delivery platforms that act through oncogene-silencing, tumor-cell killing, immune activation, or combinations of these mechanisms. Lipoplexes, lipid-based nanoparticles, microneedles, fusion peptides, and SNAs primarily carry DNA or siRNA, whereas electroporation, gene guns, DNA tattooing, and laser microporation use physical methods to overcome tissue barriers and BO-112 nanoplexes stimulate innate antitumor immunity. However, their overall clinical impact has been limited mainly by local delivery constraints, sparse human evidence, and uncertain response durability. The evidence is uneven: electroporation and BO-112 have the clearest human signals, canine lipoplex combinations provide strong translational evidence, and most other platforms remain preclinical or limited to superficial and injectable lesions.

A common pattern among the most successful strategies is the need to link tumor cell death with immune system activation in order to produce broader, systemic antitumor effects. Research conducted in immune-competent and clinically relevant models, such as spontaneous canine melanoma, provides particularly strong evidence that gene-based therapies can overcome some of the limitations seen in traditional laboratory models. Despite this progress, none of the reviewed platforms has established durable clinical efficacy as a stand-alone melanoma treatment. Overall, the literature supports these platforms most strongly as components of multimodal treatment. Future studies should prioritize systemic or multisite delivery, direct comparators, standardized quantitative endpoints, and designs that isolate each platform’s contribution within combination regimens.

REFERENCES

1. American Cancer Society. Cancer Facts & Figures 2025. Atlanta (GA): American Cancer Society; 2025.

2. AIM at Melanoma Foundation. Melanoma facts and statistics [Internet]. Frisco (TX): AIM at Melanoma Foundation; [cited 2026 Aug 4]. Available from: https://www.aimatmelanoma.org/facts-statistics/

3. Skin Cancer Foundation. Skin cancer facts and statistics [Internet]. New York (NY): Skin Cancer Foundation; [cited 2026 Aug 4]. Available from: https://www.skincancer.org/skin-cancer-information/skin-cancer-facts/

4. Davis LE, Shalin SC, Tackett AJ. Current state of melanoma diagnosis and treatment. Cancer Biol Ther. 2019;20(11):1366–1379. doi:10.1080/15384047.2019.1640032.

5. Robertson BM, Fane ME, Weeraratna AT, Rebecca VW. Determinants of resistance and response to melanoma therapy. Nat Cancer. 2024;5(7):964–982. doi:10.1038/s43018-024-00794-1.

6. Amaral T, Ottaviano M, Arance A, et al. Cutaneous melanoma: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol. 2025;36(1):10–30. doi:10.1016/j.annonc.2024.11.006.

7. Bhatia S, Tykodi SS, Thompson JA. Treatment of metastatic melanoma: an overview. Oncology (Williston Park). 2009;23(6):488–496. PMID:19544689.

8. Galanis E, Markovic SN, Suman VJ, Nuovo GJ, Vile RG, Kottke TJ, et al. Phase II trial of intravenous administration of Reolysin (reovirus serotype-3-dearing strain) in patients with metastatic melanoma. Mol Ther. 2012;20(10):1998–2003. doi:10.1038/mt.2012.146.

9. Hidai C, Kitano H. Nonviral gene therapy for cancer: a review. Diseases. 2018;6(3):57. doi:10.3390/diseases6030057.

10. Sharma D, Arora S, Singh J, Layek B. A review of the tortuous path of nonviral gene delivery and recent progress. Int J Biol Macromol. 2021;183:2055–2073. doi:10.1016/j.ijbiomac.2021.05.192.

11. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. Signal Transduct Target Ther. 2021;6(1):53. doi:10.1038/s41392-021-00487-6.

12. Finocchiaro LME, Fiszman GL, Karara AL, Glikin GC. Suicide gene and cytokines combined nonviral gene therapy for spontaneous canine melanoma. Cancer Gene Ther. 2008;15(3):165–172. doi:10.1038/sj.cgt.7701096.

13. Finocchiaro LME, Glikin GC. Cytokine-enhanced vaccine and suicide gene therapy as surgery adjuvant treatments for spontaneous canine melanoma: 9 years of follow-up. Cancer Gene Ther. 2012;19(12):852–861. doi:10.1038/cgt.2012.72.

14. Finocchiaro LME, Fondello C, Gil-Cardeza ML, Rossi ÚA, Villaverde MS, Riveros MD, et al. Cytokine-enhanced vaccine and interferon-β plus suicide gene therapy as surgery adjuvant treatments for spontaneous canine melanoma. Hum Gene Ther. 2015;26(6):367–376. doi:10.1089/hum.2014.130.

15. Chen YC, Bathula SR, Yang Q, Huang L. Targeted nanoparticles deliver siRNA to melanoma. J Invest Dermatol. 2010;130(12):2790–2798. doi:10.1038/jid.2010.222.

16. Yanagi T, Tachikawa K, Wilkie-Grantham R, Hishiki A, Nagai K, Toyonaga E, et al. Lipid nanoparticle-mediated siRNA transfer against PCTAIRE1/PCTK1/Cdk16 inhibits in vivo cancer growth. Mol Ther Nucleic Acids. 2016;5:e327. doi:10.1038/mtna.2016.40.

17. Lucas ML, Heller L, Coppola D, Heller R. IL-12 plasmid delivery by in vivo electroporation for the successful treatment of established subcutaneous B16.F10 melanoma. Mol Ther. 2002;5(6):668–675. doi:10.1006/mthe.2002.0601.

18. Daud AI, DeConti RC, Andrews S, Urbas P, Riker AI, Sondak VK, et al. Phase I trial of interleukin-12 plasmid electroporation in patients with metastatic melanoma. J Clin Oncol. 2008;26(36):5896–5903. doi:10.1200/JCO.2007.15.6794.

19. Komel T, Bosnjak M, Kranjc Brezar S, De Robertis M, Mastrodonato M, Scillitani G, et al. Gene electrotransfer of IL-2 and IL-12 plasmids effectively eradicated murine B16.F10 melanoma. Bioelectrochemistry. 2021;141:107843. doi:10.1016/j.bioelechem.2021.107843.

20. Pan J, Ruan W, Qin M, Long Y, Wan T, Yu K, et al. Intradermal delivery of STAT3 siRNA to treat melanoma via dissolving microneedles. Sci Rep. 2018;8(1):1117. doi:10.1038/s41598-018-19463-2.

21. He Y, Chen S, Song W, Wu W, Rao Z, Wang X, et al. Targeted melanoma therapy: high-efficiency siRNA delivery with R8-PEI dissolvable microneedles. Int J Pharm. 2025;675:125516. doi:10.1016/j.ijpharm.2025.125516.

22. Ruan R, Chen M, Sun S, Wei P, Zou L, Liu J, et al. Topical and targeted delivery of siRNAs to melanoma cells using a fusion peptide carrier. Sci Rep. 2016;6:29159. doi:10.1038/srep29159.

23. Aznar MA, Planelles L, Perez-Olivares M, Molina C, Garasa S, Etxeberría I, et al. Immunotherapeutic effects of intratumoral nanoplexed poly I:C. J Immunother Cancer. 2019;7(1):116. doi:10.1186/s40425-019-0568-2.

24. Rodriguez-Ruiz ME, Serrano-Mendioroz I, Garate-Soraluze E, Sánchez-Mateos P, Barrio-Alonso C, Rodríguez López I, et al. Intratumoral BO-112 in combination with radiotherapy synergizes to achieve CD8 T-cell-mediated local tumor control. J Immunother Cancer. 2023;11(1):e005011. doi:10.1136/jitc-2022-005011.

25. Márquez-Rodas I, Dutriaux C, Saiag P, de la Cruz Merino L, Castañón Álvarez E, Robert C, et al. BO-112 plus pembrolizumab for patients with anti-PD-1-resistant advanced melanoma: phase II

clinical trial SPOTLIGHT-203. J Clin Oncol. 2025;43(25):2806–2815. doi:10.1200/JCO-24-02595.

26. Steitz J, Britten CM, Wölfel T, Tüting T. Effective induction of anti-melanoma immunity following genetic vaccination with synthetic mRNA coding for the fusion protein EGFP.TRP2. Cancer Immunol Immunother. 2006;55(3):246–253. doi:10.1007/s00262-005-0042-5.

27. Leitner WW, Baker MC, Berenberg TL, Lu MC, Yannie PJ, Udey MC. Enhancement of DNA tumor vaccine efficacy by gene gun-mediated codelivery of threshold amounts of plasmid-encoded helper antigen. Blood. 2009;113(1):37–45. doi:10.1182/blood-2008-01-136267.

28. van den Berg JH, Nuijen B, Beijnen JH, Vincent A, van Tinteren H, Kluge J, et al. Optimization of intradermal vaccination by DNA tattooing in human skin. Hum Gene Ther. 2009;20(3):181–189. doi:10.1089/hum.2008.073.

29. Geukes Foppen MH, Rohaan MW, Borgers JSW, Philips D, Vyth-Dreese F, Beijnen JH, et al. Intradermal naked DNA vaccination by DNA tattooing for mounting tumor-specific immunity in stage IV melanoma patients: a phase I clinical trial. Oncol Res Treat. 2024;47(7–8):351–359. doi:10.1159/000537896.

30. Scheiblhofer S, Thalhamer J, Weiss R. Laser microporation of the skin: prospects for painless application of protective and therapeutic vaccines. Expert Opin Drug Deliv. 2013;10(6):761–773. doi:10.1517/17425247.2013.773970.

31. Weiss R, Hessenberger M, Kitzmüller S, Bach D, Weinberger EE, Krautgartner WD, et al. Transcutaneous vaccination via laser microporation. J Control Release. 2012;162(2):391–399. doi:10.1016/j.jconrel.2012.06.031.

32. Zheng D, Giljohann DA, Chen DL, Massich MD, Wang XQ, Iordanov H, et al. Topical delivery of siRNA-based spherical nucleic acid nanoparticle conjugates for gene regulation. Proc Natl Acad Sci U S A. 2012;109(30):11975–11980. doi:10.1073/pnas.1118425109.