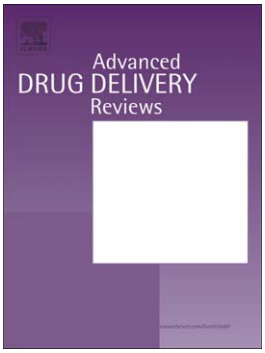


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# Targeted Drug Delivery to Melanoma

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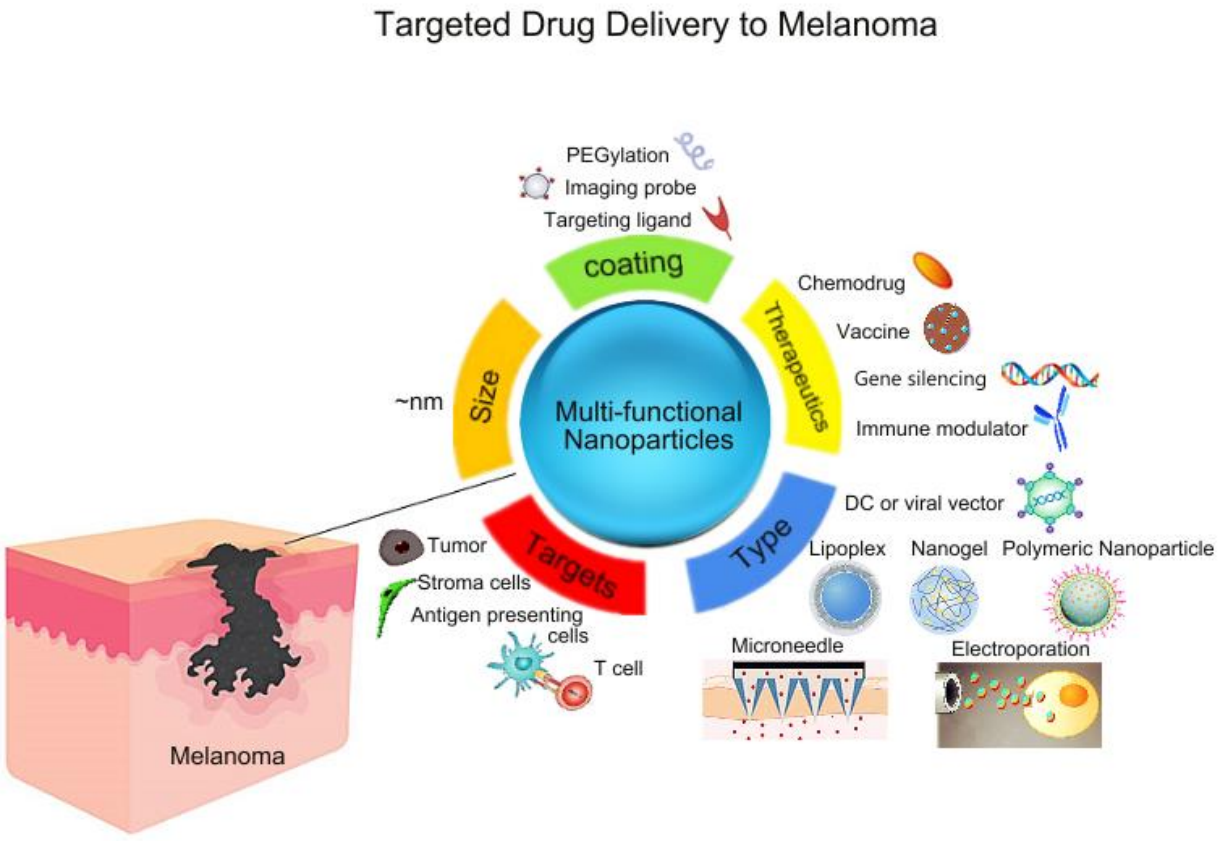
## Abstract

Melanoma derived from melanocytes is the most aggressive genre of skin cancer. Although the considerable advancement in the study of human cancer biology and drug discovery, most advanced melanoma patients are inevitably unable to be cured. With the emergence of nanotechnology, the use of nano-carriers is widely expected to alter the landscape of melanoma treatment. In this review, we will discuss melanoma biology, current treatment options, mechanisms behind drug resistance, and nano-based solutions for effective anti-cancer therapy, followed by challenges and perspectives in both pre-clinical and clinical settings.

**Key words**: melanoma, nanoparticles, targeted drug delivery

### Graphical abstract



# 1. Introduction

Melanoma is one of the commonest forms of skin cancer, with an estimated 76,380 new cases in the United States in 2016 [1]. Melanoma incidence is not strongly correlated with age; moreover, it is one of the most general causes of cancer and cancer deaths in people aged 20–35. Overall, melanoma is a strong example of how genetics and the environment cooperate to stimulate melanoma-genesis [2]. Early-stage melanoma is curable by surgical resection; however, metastasis results in poor prognosis, with five-year survival rates dropping from 98 % to 17 %. This is largely due to metastatic melanoma being largely refractory to conventional therapeutic interventions. Hence, new therapeutic strategies are deemed necessary.

Early detection of melanoma is important, as the five-year survival rate of patients without metastatic disease is 90 %. Further, metastases-bearing melanoma patients often show a poorer prognosis of only 10–20 %, with an overall median survival of only 6 to 9 months [3]. The available treatment options are much more limited for metastatic stage patients because metastatic melanoma is noted for its high drug resistance. However, early melanoma detection is hindered by the lack of appropriate tumor markers and public education, and also absence of clinically significant symptoms until the disease reaches an advanced stage [4].

Currently, there are five types of standard treatment for melanoma patients, including surgery, radiation therapy, chemotherapy, immunotherapy, and targeted therapy [5]. In the study of new targeted therapies, proto-oncogene protein B-raf (BRAF) and mitogen-activated protein kinase (MEK) specific inhibitors have emerged with distinct survival benefits. Despite ongoing advancement in the study of metastatic melanoma, emerging drug resistance and systemic toxicity confront efficacy. Overall, the success rate for the treatment of melanoma is relatively low compared with other cancer types.

Facing the challenges of off-target effects, serious toxic adverse effects, and short circulation time in conventional systemic drug administration, researchers have developed nanoparticle (NP) technology as a means of overcoming these disadvantages. Over the past few years, significant advances in NP-based drug delivery have made it easier for researchers to develop effective treatments. Because NPs offer excellent barrier protection to avoid host immune system attack and enzymatic degradation, immune-modifiers can be administrated to downregulate oncogenes or restore tumor suppressor microenvironment for more effective cancer chemotherapy, thus combating drug resistance. In this review, we will go through NP-based strategies for effective transdermal delivery of therapeutically payload in treating melanoma.

# 2. Current pre-clinical study of melanoma

## 2.1 Biology of melanoma

Melanoma is a skin cancer that is derived from melanocytes, a type of pigment cell. Melanocytes are located at the bottom of the skin epidermal layer and are responsible for generating melanin, which is the pigment responsible for a “suntan” and protects the skin against damage from ultraviolet radiation exposure. In general, people with pale skin color (i.e., Caucasians) have an increased risk of melanoma-genesis comparing to darker skin populations (i.e., Africans, East Asians, and Hispanics) [6]. Similarly, people who have excessive sun exposure are at higher risk for tumorigenesis.

The development of melanoma is a multistep process with clinical and histological characteristics [7]. Melanoma-genesis can be histologically divided into five stages. In stage one, acquired nevi form as a result of increased melanocyte proliferation. Nevi are benign skin lesions; however, the majority of malignant melanomas are derived from nevi [8]. In stage two, melanocytes grow into dysplastic nevi showing abnormal differentiation. In stage three, dysplastic nevi continue developing into the radial

growth phase (RGP) primary tumor. RGP melanomas develop within the epidermis but do not have the ability to invade into the dermis. In stage four, RGP melanomas acquire invasive potential through genetic alterations and invade into the dermis. This is the so called vertical growth phase (VGP). At this phase, melanomas possess the potential of self-sufficient growth signals and the ability to invade, thus making treatment options more limited. In the fifth and last stage of melanoma development, the metastatic lesion is formed. In this stage, VGP melanomas continue to grow larger and invade surrounding tissues. Metastatic lesions form in distant organs the melanoma becomes metastatic.

During the past few decades, epidemiological studies have identified several melanoma risk factors. These factors include excessive ultraviolet (UV) light exposure, moles (nevi), family history of melanoma, and a weakened immune system [9].

## 2.2 Oncogenic pathways

Melanoma cells develop multiple unique signaling pathways in regulating tumor proliferation, migration, cell differentiation, as well as apoptosis. Downregulated signaling pathways often lead to tumorigenesis for melanoma development. Signal pathways can be activated by external stimuli and function to convey a signaling cascade from the cell surface to intracellular downstream effectors or to be activated by constitutively activated internal oncogenes without external stimuli. Deregulated cell proliferation and apoptosis are two major common factors required for most of the human malignant tumor development that is mediated by oncogenic signaling pathways.

The N-RAS oncogene is mutated at codon 61 in 20% of melanomas [10]. Most mutations result in the constitutive activation of the N-RAS oncogene that then impairs GTP hydrolysis [11]. Constitutively active N-RAS activates the BRAF/MEK/ERK (MAPK) and phosphoinositide 3-kinase/ Protein kinase B (PI3K/AKT) cascades that further facilitate the proliferation, evasion, and metastases of tumor cells (**Figure 1**).

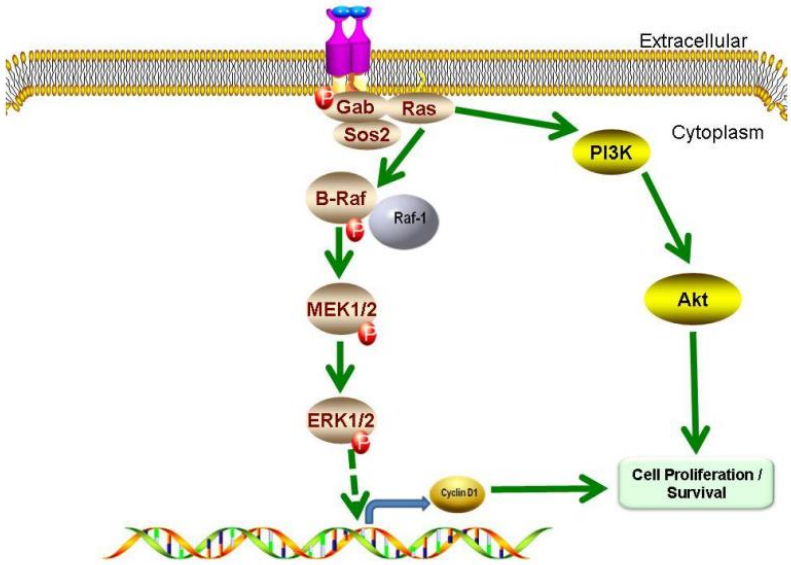



**Figure 1**. Melanoma oncogenic pathways. Schematic depicting major pathways which induce melanoma development.

### A. MAPK and ERK1/2 pathways

The mitogen-activated protein kinase (MAPK) pathway is often referred to as the extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway for its growth factor receptor-mediated (i.e., Epidermal growth factor receptor (EGFR-), Fibroblast growth factor receptor (FGFR-), or Platelet-derived growth factor receptors (PDGFR) -mediated) activation initiated from the plasma membrane [12].

Once activated, the MAPK pathway would facilitate a series of signaling cascades, including RAS/RAF/MEK/ERK. It begins with a growth receptor receiving stimuli and then activating RAS by converting inactive RAS-GDP to active RAS-GTP [13]. The active RAF-GTP recruits RAF, and activates RAF by phosphorylation specific to the cell membrane. Phosphorylated RAF then phosphorylates MEK1/2, which then activates ERK1/2 by phosphorylation. Activated ERK1/2 signaling pathway boosts cell proliferation by conveying signaling through protein phosphorylation to cytoplasmic and nuclear effectors. The phosphorylation results in fast cell proliferation by regulating Cyclin D1, p21, p27, and c-myc [14]. The classic MAPK/ERK1/2 pathway needs extracellular stimuli to initiate activation of the signaling cascade. However, in melanoma and other human cancers, including thyroid and colorectal cancers, constitutively activated ERK1/2 signaling results from the BRAF (V600E) mutation could promote tumorigenesis [15, 16].

BRAF, also referred to as the proto-oncogene B-RAF or V-RAF murine sarcoma viral oncogene homolog B1, is a serine/threonine-protein kinase. B-RAF, A-RAF, and C-RAF (also known as RAF-1) constitute the RAF kinase family [17]. Through a BRAF mutation, the MAPK pathway is activated in ~7 % of human carcinomas, with approximately 60 % of cutaneous melanomas having activating mutations [18]. A BRAF mutation is very common in cutaneous melanoma; its incidence is very rare in acral, mucosal, conjunctival, and uveal melanomas [19, 20]. In 90 % of melanoma tumors, the BRAF mutation is a single-base missense from T to A; this would change valine to glutamic acid at codon 600 (V600E) in exon 15 [21]. Mutated BRAF (V600E) protein is highly activated comparing to wild-type, owing to a conformational transform in protein structure, where glutamate phosphorylation occurs at the thr598 and ser601 phosphorylation sites [22].

Although the BRAF (V600E)-initiated, constitutively-activated ERK1/2 pathway contributes to increased cell proliferation for tumor development, recent reports indicate hyper-activated ERK1/2 activity at a level that could lead to cell senescence for nevi formation without melanoma formation in transgenic mice [23]. Previously, other groups have indicated that BRAF (V600E) is critical to promoting melanocyte proliferation for the formation of benign nevi, but it is not the only key factor for melanoma development [24]. Collectively, it is accepted that more than just a BRAF (V600E) mutation is required for melanoma development.

**B. PI3K/AKT pathways**

The PI3K/AKT pathway participates in fast cell proliferation, and also drug resistance [25]. The PI3K/AKT pathway is activated by extra-cellular stimulation of receptor tyrosine kinases. The activated AKT then trans-locates to the cytoplasm or nucleus to activate downstream effectors for different signaling cascades. During the process of activation, PI3K/AKT signaling can be inhibited by PTEN (phosphatase and tensin homologue deleted on chromosome 10) [26].

There are three AKT family members, AKT1, AKT2, and AKT3, known as important downstream effectors that relay the signal transduction cascade coming from PI3K. AKT3 plays major role in melanoma-genesis. Earlier immunohistochemical studies found that ~70 % of cutaneous melanomas have elevated AKT expression compared to normal melanocytes [27]. Further, previous studies have shown that, of the three AKT family members, major AKT3 activation facilitates tumor progression. Inhibition of AKT3 using small (or short) interfering RNA (siRNA) leads to decreased melanoma development [28].

## 2.3 Animal models in melanoma research

More advanced pre-clinical melanoma models have been developed that assemble the relevant clinical conditions. To gain a broader understanding of tumor biology, these in vivo models mirror true melanoma settings. The most widely used pre-clinical model is the murine models, including but not limited to xenograft, syngeneic, and genetically engineered models [29, 30].

**A. Xenograft models**

Xenograft models are built upon the inoculation of human melanoma cells into an immune-deficient murine model. Once subcutaneously implanted into immune-deficient mice, melanoma cells proliferate and metastasize along lymphatic tissue and blood vessels, which strongly resemble human conditions [31]. Studies based on xenograft models mainly focus on tumor growth mechanisms, major tumorigenesis pathways, pharmaceutical therapy, bio-availability and toxicities.

Unfortunately, cultured melanoma cell lines are purified clones that differ from the original parental patient-derived cells. They may lose certain metastasis promoting markers while proliferating under subcutaneous microenvironment. This results in irrelevant predictions of clinical outcome and explains most clinical trial failures [32]. Patient derived tumor can be xenograft directly into animal models, but the expenses of model establishment and maintenance are rather high, comparing to purified cell lines.

**B. Syngeneic allograft**

Syngeneic models are developed by inoculation of melanoma cells into the same species and genetic background [33]. These mice are immune-competent with fully functional immune system. In the study of melanoma microenvironment, dendritic cells (DCs) presenting with tumor released antigens, allowing natural interactions between melanocytes and immune cells, such as T cells and B cells [34].

Several types of cells have been applied to syngeneic transplantations. The most commonly utilized cell types were building upon C57BL/6J mice, which were induced by specific chemical reagents. This cell line, so called the B16 cell line, is characterized by a variety of propensities to tumor growth, invasion, and metastasis. The two well-established sub-clones by in vivo passaging are B16F1 and B16F10 cell lines. B16F1 has the notable distinguishing feature of low metastatic potential and can therefore be used to study the growth of primary tumors [35]. In contrast, B16F10 usually shows higher metastatic ability to distant organs, with the highest probability of metastasis in the lungs [36]. Due to its rapid growth and high turnaround rate, B16 models are perfect for animal in vivo studies. For instance, subcutaneous tumors usually reach applicable status within two to four weeks [37].

The B16 model brought valuable insights into melanoma immunology studies, as well as immunotherapy strategies, however, when compared with human melanoma, the adhesion proteins and growth factors of mouse cell lines are quite different. Despite the B16 cells being able to produce a variety of sub-clones, they come from a unitary inbred mouse of same origin, thus unrepresentative of human conditions [38, 39]. Scientific interpretations based on such model can be misleading.

**C. Genetically engineered models (GEMs)**

Genetic engineering models built upon transgenic mice with engineered gene expression specific to melanoma-genesis [40]. We have gain tremendous understanding of gene functions through studies of GEMs for effective targeting therapy. By combining with other neoplasm-inducing strategies such as UV-induction, melanoma development is more accurately assessed. Compared with other pre-clinical models, GEMs are more precise in predicting drug efficiency [41].

**RAS model**. It has been found that RAS family proteins contain highly mutations in cutaneous melanoma [42]. Their specific mutations have been investigated in depth in murine models, in order to explain underlying mechanism of melanoma-genesis. **PTEN/BRAF models**. Researchers have found that 65% of

malignant melanoma cells carry somatic missense BRAF mutations [43]. In most malignant melanoma cases, BRAF mutations and RAS mutations are mutually exclusive, where MAPK signal transduction is excessively activated. The silencing of PTEN will further induce excessive activation of AKT signal pathway, thus up-regulating BRAF gene expression to be activated [44]. **RET model**. This model is established upon the RET proto-oncogene, which encodes for glial cell-derived neurotrophic factor-specific receptor tyrosine kinase [45]. RET gene expression can cause the progressive growth of melanoma. As a result, benign melanoma tumors occur months later, following by eventual malignant tumor growth and organ metastasis. During tumorigenesis, the MAPK signaling cascades are high activated, where the expression of RET transgene is found to increase in a gradual manner.

Although GEMs models are highly applicable, we are still challenged with multiple limitations. Genetic modified murine strains accompanied with significant labor costs, and the expenditure is rather high. In addition, some genetic alterations have adverse effects on reproductive ability, thus dampen the effective genotyping for targeted therapy.

**D. Physical or chemically induced models**

Induced by UV radiation (UVR), these models could highly assemble natural human melanoma-genesis. But the drawbacks of murine models are evitable comparing to human conditions. For one, human skin and mouse skin melanoma cells reside in different locations [46]. Human melanocytes are mainly located in the basal layer of the cuticle and the epidermal dermis junction; therefore, they are vulnerable to be invaded by UVR. However, in murine models where melanocytes are located in the deep dermis and are well protected, there is less chance of natural occurrence of melanoma [47]. 7,12-Dimethylbenz[a]anthracene (DMBA) and 12-O-Tetradecanoylphorbol-13-acetate (TPA) can be utilized in situ, in order to stimulate melanoma-genesis [48, 49], but tumor cells induced contain no melanin pigments, thus less representative of natural settings.

Researches based on different murine models provide significant insights and valuable interpretations of melanoma development. By comparing respective advantages and disadvantages of each model, great progressions have been achieved. The studies of varies pre-clinical models are of great translational value in the diagnosis, treatment, and prevention of melanoma. Although currently incurable, increasing understating of disease biology will offer more effective treatment options for patients with advanced melanoma.

## 3. Current treatment of melanoma

The first-line treatment options for melanoma patients are surgical removal and radiation therapy. Surgery can involve wide local excision, lymphadenectomy, and sentinel lymph node (LN) biopsy. In many cases, surgical removal of melanoma can be combined with chemotherapy, radiation therapy, biologic therapy, and targeted therapy [50]. Radiation therapy uses high-energy radiation to induce melanoma cell death. Depending on the site, radiation can be classified into two categories, external and internal [51]. An external source of radiation can be used to direct high-energy beams to the tumor with external therapy versus an internal therapeutic approach where radiation is targeted to internal metastatic melanoma using wire needles or catheters [52].

Unfortunately, metastasis results in poor melanoma prognosis. Metastatic melanoma is aggressively resistant to chemotherapeutic regimes. Many studies on the molecular basis of melanoma survival and proliferation have identified apoptotic resistance of melanoma cells as the underlying cause of chemo-resistance [53]. This presents a formidable challenge in devising treatment strategies for advanced melanoma, and until recently there was little advancement in standards of care. Dacarbazine has been the sole first-line treatment for melanoma since its US Food and Drug Administration (FDA) approval in 1976. It has demonstrated a response rate of 10–20 % in Phase I and Phase II clinical trials, but the

benefit in overall survival (OS) has never been clearly established [54-57]. Interferon alpha (IFN-α), a type I interferon, is used for adjuvant immunotherapy in advanced melanoma; however, improvements in OS are debatable, and the clinical markers for the subset of patients' sensitive to the adjuvant therapy have not been identified [58, 59]. High-dose Interleukin-2 (IL-2) was approved in 1998, but, again, the response rate is only about 10 %, and therapy involves grade 3 toxicities [60].

Advancement in understanding of cancer progression and survival has resulted in a resurgence of interest in developing newer therapeutic interventions in recent years [61]. Identification of driver oncogenic mutations in serine/threonine kinase BRAF, a critical functional component in the RAS-RAF-MEK-ERK-MAP kinase cascade, provided unique opportunities in the treatment of malignant melanoma [18, 62]. Vemurafenib and dabrafenib, two structurally unrelated inhibitors selectively targeting V600E, a missense mutation, that constitutes about 65 % of all malignant melanomas, resulted in improvement of Disease free survival (DFS) and OS, leading to regulatory approval in 2011 and 2013 [63, 64]. However, this strategy can only address melanomas driven by the activating V600E mutation and suffers from resistance mechanisms driven by reactivation of the MAPK pathway, often paradoxically induced by the inhibitors [65, 66]. Combined inhibition of BRAF and MEK can reduce disease progression risk by 25 % over BRAF inhibition alone and delay development of resistance, but it cannot overcome it altogether [67].

Immunotherapeutic strategies have been extensively investigated against melanoma in recent years. Tremendous excitement was generated as the "checkpoint inhibitors" demonstrated improvement in OS and DFS over conventional chemotherapy regimens [68]. Ipilimumab, a monoclonal antibody targeting cytotoxic T-lymphocyte antigen 4 (CTLA-4), received FDA approval in 2011, followed by approval of pembrolizumab and Nivolumab, antibodies against programmed cell death 1 (PD-1), in 2014 [69-72]. However, new challenges rapidly emerged as a high proportion of patients demonstrated transitory or no responses against checkpoint inhibitors, while long-term survival and cure was further achieved in a small subset of patients [73]. It is thus crucial to identify the right patient subset that may benefit from immunotherapy however no biomarker can currently predict clinical outcomes [74].

This segment of the review will focus on strengths and weaknesses of current therapeutics, with emphasis on novel modalities that are currently being explored in the clinic, and the challenges that hinder the battle against advanced melanoma.

### 3.1 Chemotherapy: Single arm and in combination

The CTLA-4 monoclonal antibody Ipilimumab and the BRAF kinase inhibitor vemurafenib changed the treatment landscape of metastatic melanoma. However, chemotherapy is still a relevant tool to clinicians because the majority of patients do not respond to immunotherapy, and, further, not every patient carries the V600E mutation on BRAF. Even if a patient harbors the mutation and can be treated with the kinase inhibitor, drug resistance develops rapidly, and survival benefit is not significant over the long term.

Dacarbazine has been the standard of care for management of metastatic melanoma ever since its regulatory approval in 1976. The drug has a response rate of about 10–20 %; however, its OS benefit had never been validated in a randomized Phase III clinical trial [55]. Temozolomide, an analog of dacarbazine, was not found to provide a significantly better response rate or OS when compared with dacarbazine in a European Phase III trial [57]. Apart from alkylating agents, other cytotoxic classes of drugs, like nitrosoureas, microtubule toxins, and taxanes, have been investigated in melanoma [75-78]. These agents provided no significant OS benefit over dacarbazine. DNA-crosslinking agents like cisplatin did not demonstrate a promising effect in melanoma. Responses varied widely from 10–50 % [79]. In one randomized Phase II trial, cisplatin was combined with WR-2721, a chemoprotective agent for normal tissues against radiation therapy, alkylating agents, and platinum compounds [80]. Cisplatin had a response rate of 16.3 % in single-arm treatment against a rate of 23.3 % in the combination arm. However,

toxicity was not mitigated and rather was enhanced with the combination regimen, and no additional OS benefit was presented.

Immunological agents have been combined with chemotherapy, but the results have been less than satisfactory. IFN-α and IL-2 have been explored in combination with chemotherapeutic regimes in multiple clinical trials. Meta-analyses revealed that while the combination of immunological agents and cytotoxic drugs can significantly improve response rates, it provides no survival benefit [81]. Further, the toxicity in the combination regimens dampens the improvement in response. The combination of IFN-α with chemotherapy drugs in particular was found to be associated with hematological toxicities [82].

Angiogenesis has been established as one of the well-defined processes for tumor proliferation and survival [83]. Vascular endothelial growth factor (VEGF) facilitates angiogenesis [84], and chemotherapeutic resistance of metastatic melanoma is rendered, in part, by VEGF overproduction [85]. Hence, combining chemotherapeutics with angiogenetic inhibitors, like monoclonal antibodies targeted against VEGF, is a clinically significant strategy. A combination regimen of paclitaxel, carboplatin, and bevacizumab, a monoclonal antibody targeting VEGF, on patients with Stage IV melanoma and not qualified for surgery has been explored in a Phase II clinical trial [86]. The median progression-free survival (PFS) data was about 6 months, while the median OS was about 12 months. A similar trial investigated the combination of temozolomide and bevacizumab against a combination regimen of nab-paclitaxel, carboplatin, and bevacizumab [87]. Patients on the latter regime had a better PFS rate at 6 months (52.1% vs. 32.8 %); however, that did not translate into higher OS (13.9 months vs. 12.3 months). There is at least one other trial that explored temozolomide and bevacizumab in chemotherapy-naïve patients and recorded a significantly higher OS in patients harboring the V600E BRAF mutation (12 months vs. 9.2 months). Researchers attempted to address chemotherapeutic resistance mediated by apoptotic resistance through a combination of chemotherapy with an antisense oligonucleotide against B-cell lymphoma 2 (Bcl-2) [88]. Oblimersen, a Bcl2 antisense oligonucleotide, was developed and investigated in combination with dacarbazine. No OS benefit was observed in the overall population, although the benefit was significant in patients with normal lactate dehydrogenase (LDH) levels [89]. However, no significant benefit in a subpopulation of patients with low-normal LDH levels was determined in further studies [90].

### 3.2 Targeted therapy

The idea of targeted therapy against cancer is focused around the targets on which malignant cells have to rely for progression, survival, and proliferation. Hyper-activated pathways provide a therapeutic opportunity because progression of cancer has a higher dependence on these pathways over normal cells. Kinases, phosphatases, and proteases are reasonable tools worthy of clinical investigation, because rationally designed drugs can bind selectively on active sites and potentially mediate a therapeutic effect [91, 92]. NRAS and BRAF mutations are not simultaneously presented and implicated to drive pathogenesis in metastatic melanoma through the same pathway [93].

Ras farnesyl transferase inhibitors have been one of the earliest classes of drugs investigated in clinical trials; however, results have been generally disappointing [94, 95]. Although tissue analyses showed potent target inhibition in advanced melanoma, no tumor response was demonstrated in a Phase II trial [96]. Sorafenib, a broad-spectrum kinase inhibitor targeting both CRAF and BRAF, demonstrated a modest response of about 30 % when investigated in combination with carboplatin and paclitaxel; however, responses were disappointing as a monotherapy [97]. The initial clinical success of vemurafenib and dabrafenib targeting BRAF (V600E), with 50 % response rates, was a major breakthrough in the management of metastatic melanoma [63]. However, initial excitement was rapidly replaced by disappointment as a majority of patients suffered relapse, and molecular analyses revealed multiple pathways of acquired resistance, primarily by compensation from other pathways [98]. Reactivation of MAPK and ERK has been demonstrated as a clinical marker of resistance development [99]. Trametinib,

an MEK inhibitor, has been investigated in combination with BRAF inhibitors, and although PFS improves to 9–10 months, resistance development cannot be prevented in the long run [100, 101]. The mechanism underlying resistance is not clearly understood, although exome sequencing on a small number of patients revealed an activating mutation on MEK2 [102]. The potential of selective ERK inhibitors has been harnessed to address resistance against BRAF inhibition; however, this approach suffers from the actuation of an ERK inhibition-mediated negative feed, leading to RAS and PI3K signaling [103, 104]. Recently, the role of the tumor microenvironment in resistance-acquired, post-BRAF inhibition was suggested by Hirata et al. They demonstrated that BRAF inhibition triggers MAPK signaling in melanoma-associated fibroblasts (MAFs), subsequently leading to kinase integrin/focal adhesion kinase (FAK) signaling and increasing tolerability of melanoma cells against BRAF inhibition. A BRAF and FAK inhibitor combination prevented ERK reactivation and improved tumor control, although a complete remission was not observed when investigated in preclinical models [105].

MEK and ERK reactivation, although common, is not the sole driver of resistance to a combination BRAF/MEK inhibitor, and receptor tyrosine kinase overexpression has been routinely observed to induce compensation by PI3K-AKT pathways [106, 107]. However, targeting the PI3K-AKT pathway is difficult because mTOR kinase inhibition induces reactivation of AKT signaling by feedback loops, and effective targeting becomes challenging [108, 109].

About 15–20 % of melanomas harbor an NRAS mutation, and while there is an active interest in developing targeted therapies against BRAF mutation, successful therapies against NRAS mutant melanomas are an unmet medical need. NRAS mutant melanomas signal primarily through CRAF and not BRAF, and induction of MAPK signaling is triggered when treated with inhibitors targeting BRAF mutation [110, 111]. The MAPK signaling cascade is still critical to NRAS mutant melanomas. Mono-therapy with MEK inhibitors like trametinib or selumetinib has been modest, and finding combinatorial additive therapies targeting downstream of NRAS is critical [112, 113]. Overall, targeted therapies have been clinically impactful for melanoma management, although they suffer from the drawback of resistance development after initial response or a lack of translation of target inhibition into disease control. The future of targeted therapies in melanoma management rests on successful translation of the understanding of the biological mechanisms of resistance into clinically significant therapeutic combinations.

### 3.3 Immunotherapy

The clinical success of checkpoint inhibitors changed the landscape of melanoma research, and there is considerable interest in understanding the immunology of melanoma and translating it to robust therapeutic strategies. The classic two-signal activation model was formulated out of basic research on understanding T-cell activation that involved the contribution of both antigens and secondary stimuli [114]. The co-inhibitory receptors or the immune checkpoints like CTLA-4 and PD-1 promote downregulation by preventing T-cell activation [115, 116]. Hence, negative regulatory mechanisms are a major hurdle in the T-cell response to tumors. The T cells may undergo functional inactivation and death in the tumor microenvironment, because PD-1 expressed on T cells engages with cancer cells (which would express Programmed death-ligand 1 (PD-L1)) [117]. Hypothesizing that the blockade could break tolerance and rescue the immune response, researchers developed monoclonal antibodies targeting the immune checkpoints.

#### 3.3.1 Immune checkpoint inhibitors

Ipilimumab was the first major clinical success of a checkpoint inhibitor that functions by binding to CTLA-4, thereby actuating down-regulation of the T-cell response. It received regulatory approval after demonstrating OS benefits in patients treated previously with chemotherapeutic regimens or IL-2 and also in untreated patients. In one of the earliest trials, melanoma patients in Stage III or Stage IV who were not

eligible for resection demonstrated a 10-month OS when treated with Ipilimumab in combination with gp100 peptide vaccine, against an OS of 6.4 months on vaccine single-arm therapy [69]. Interestingly, a recent meta-analysis revealed that about 20 % of patients with advanced melanoma may have long-term survival benefits, indicating the possibility of remission in a subset of patients [73]. The next-generation antibodies targeting the PD-1/PD-L1 axis entered into clinical trials following Ipilimumab and pembrolizumab, a monoclonal antibody against PD-1, received accelerated approval in 2014. Pembrolizumab was compared against chemotherapy in patients non-responsive to Ipilimumab, and the 6-month response rate was approximately twice that of the chemotherapy arm [70]. Nivolumab, another antibody targeting PD-1, was compared against investigator's choice chemotherapy (ICC) and demonstrated objective response in 31.7 % of the patients, as opposed to 10.6 % in the ICC arm [118]. However, as with kinase inhibitors, the shortcomings of immune checkpoint inhibitors were quickly revealed because a large population of patients did not respond to therapy, while no bio-marker could be identified for patients who received long-term benefits.

With the success of mono-therapies, the exploration of CTLA-4 and PD-1 checkpoint inhibitors as a combination regimen was the next rational step. CTLA-4 and PD-1 are believed to have distinct regulatory roles, acting in different stages of T-cell activation. Targeting both checkpoints induces non-reductant changes in gene expressions and demonstrates a synergistic interaction when combined [119, 120]. The combination of Nivolumab and Ipilimumab has been demonstrated to provide a longer PFS benefit (11.5 months overall and 11.7 months in BRAF-mutant melanoma patients) in comparison to Nivolumab or Ipilimumab alone, which offered a PFS of 6.9 and 2.9 months, respectively [121]. This was comparable to a dabrafenib and trametinib combination in melanoma patients with BRAF mutation (9.3–11.4 months) [101]. The trial further observed a similar PFS with Nivolumab or Nivolumab combined with Ipilimumab in patients who were positive for PD-L1. It will be worthwhile to look at long-term survival and investigate if PD-L1 expression can be exploited as a clinical bio-marker to predict whether a particular patient is suitable for mono-therapy or combination therapy. Currently, other co-inhibitory receptors like Lymphocyte-activation gene 3 (LAG-3), mucin-domain containing-3 (TIM3), and T cell immunoreceptor with Ig and ITIM domains (TIGIT) are being explored in clinical trials [122].

### 3.3.2 Therapeutic vaccines

A strong association between tumor-infiltrating cytotoxic T lymphocytes (CTL) and patient survival drove interest in the development of vaccine strategies [123]. However, initial clinical trials did not offer any survival benefit, and in hindsight, this was primarily due to a lack of rational strategies [124]. Most therapeutic vaccines were aimed at induction of response against tumor-associated antigens (TAAs). Effective anti-tumor response, however, requires presentation of TAAs to T cells after distinct activation and maturation signals are received by antigen-presenting cells. Further, the activated T cells have to expand, travel to tumor sites, and infiltrate the immunosuppressive tumor microenvironment to be able to recognize and kill tumor cells. Some of the initial trials investigated free peptide antigens with poor pharmacokinetic profiles, administered without a delivery system or an immuno-stimulatory adjuvant, which contributed to failure and generated cynicism about the future of vaccines as an effective therapeutic strategy [125]. As additional knowledge has been acquired about the immunology of cancer, the current focus has shifted to combining vaccines with other immunomodulatory agents. A melanoma peptide antigen vaccine (gp100) was investigated in combination with IL-2 and demonstrated a response rate of 16 % over 6 % and a median PFS of 17.8 months over 11.1 months. Metastatic melanoma patients were treated with DCs stimulated with an assortment of melanoma antigens, and the survival benefit in the immunized group was 13.6 months, over 7.3 months in the control group. However, as with most immunotherapies, only a subset of patients who were immunized responded to the therapy, as demonstrated by a positive CTL response, extending to a longer survival benefit (21.9 months vs. 8.1 months). Recently, a tumor vaccine that secretes Granulocyte-macrophage colony-stimulating factor (GM-CSF) was in Phase III clinical trial. With the help of Ipilimumab, these patients bearing Stage III or

Stage IV melanoma showed longer overall survival (17.5 months vs. 12.7 months). Unfortunately, PFS was not clearly extended [126].

### 3.3.3 Emerging directions in immunotherapy

A few other active immunotherapy approaches involving T cells are currently generating interest in academic labs and clinics. Adoptive T-cell therapy is one of the most personalized and effective treatment methods available for management of metastatic melanoma, involving proliferation of tumor-infiltrating lymphocytes (TILs) ex vivo and transferring the TILs back to the patient augmented with other immuno-modulators like vaccines [127]. When combined with lympho-depletion, objective response rates can be dramatic and reach 49–72 % in metastatic melanoma patients, further providing durable survival benefits over the long term [128, 129]. However, one of the major hurdles that limit this otherwise effective treatment is the economic cost and skilled labor associated with this complex, personalized therapy [130]. Chimeric antigen receptors (CARs), a class of engineered fusion proteins combining an antibody-derived antigen recognition domain and a signaling domain, can bypass immune escape exploited by malignant cells by their Major histocompatibility complex (MHC)-independent recognition of TAAs [131]. However, major clinical success of this modality is restricted to hematological cancers like multiple myeloma, and its impact on solid tumors is yet to be clinically validated [132].

BRAF inhibition and combination BRAF/MEK inhibition had been reported to be associated with higher CD8+ T-cell infiltration and PD-L1 expression [133]. Hence, a combination of MAPK signaling inhibitors with immune checkpoint inhibitors could be considered a critical therapeutic strategy. Treatment with BRAF inhibitors induces an increase in antigen expression and decrease of immunosuppressive factors [134]. The hypothesis is that the antigen-presenting cells would pick up antigen released from dying melanoma cells and cross-present them to T cells, and hence combination with checkpoint inhibitors can potentially augment T-cell response. However, there are numerous limitations to this approach. Autoimmune toxicity is only one of them. In fact, one of the early trials combining vemurafenib and Ipilimumab had to be terminated due to liver toxicity issues [135]. Efforts need to be focused to understand the immunological modulations followed by treatment with combinations of targeted therapies and immunotherapies and translate the knowledge into tailoring the appropriate dosage and schedule of therapeutics in subsequent clinical trials.

## 3.4 Demand of efficient delivery systems: Scope of nanomedicine

Nanomedicine involves the development and design of materials at the nanoscale and has been extensively investigated in the past few decades for development of efficient delivery systems for diagnostics and therapeutics in a multitude of diseases [136]. Resistance to chemotherapeutics in melanoma has been attributed to high intratumoral pressure induced by stromal cells, poor perfusion, drug efflux, and intracellular entrapment, leading to inefficient drug delivery [137]. It is theoretically possible to design NP formulations capable of altering bio-distribution of therapeutic cargo and mediating better payload accumulation in a target of interest by active targeting [138, 139].

There are many arenas in which NPs can potentially serve as robust delivery platforms. With increasing demand for combinatorial drug regimens, it is possible to harness the potential of NPs to precisely tailor the ratio of individual drugs and further mediate sequential release [140-142]. Guo et al. demonstrated sensitization of melanoma cells to cisplatin therapy by co-delivery with rapamycin, an mTOR inhibitor that mediated microenvironment modulation and synergistically affected the efficacy of cisplatin [143]. Peptide antigens, when delivered systemically, suffer from suboptimal cytosolic delivery to DCs, and high-dose administration may induce significant toxicity. Xu et al. managed to co-encapsulate Trp2, a melanoma antigen with immune-stimulant CpG oligonucleotides (ODN), on a lipid-calcium phosphate NP platform and induce a potent CTL response and superior tumor inhibition in a murine melanoma model [144]. RNA interference is another key therapeutic application where NPs can serve as a potent

delivery platform. Beloor et al. explored the potential of a polymer-based siRNA delivery platform to efficiently co-deliver a cocktail of siRNAs like Bcl-2, VEGF, and c-myc in a mouse xenograft tumor model and demonstrated robust tumor control [145]. The Trp2 vaccine discussed earlier was less effective in a late-stage melanoma model. In a follow-up study, a liposome-protamine-hyaluronic acid-based NP platform was exploited to deliver siRNA against transforming growth factor-β (TGF-β), because immunosuppressive cytokines like TGF-β were found to be elevated in a tumor microenvironment. Thus, the combination of an antigen-specific CTL response was harnessed to a rational modulation of an immune microenvironment using an NP-based delivery system.

Despite intensive pre-clinical development, success in the clinical setting has been disappointing so far due to rapid reticuloendothelial system (RES) clearance, scalability, and toxicity issues [146]. The grand diversion between animal models and human diseases has further affected successful translations [147]. A considerable number of "multi-functional" NPs are currently under investigation in pre-clinical models and early clinical trials, although there is a long way to go before translation into a clinical modality.

# 4. Transdermal drug delivery to melanoma

## 4.1 Versatile delivery: Targeting strategy

Although most investigations have focused on delivering medicinal molecules directly to melanocytes, a few of them have achieved better effects in an advanced melanoma model. Thus, more and more studies have explored the therapeutic potential of other target sites associated with melanoma [148]. In clinical practice, the immune system in cancer patients has always been weakened by tumor invasion and chemotherapy, and the understanding of immunotherapy in recent years has become a promising strategy for cancer treatment and immune system reconstruction [61].

Among those immunotherapies, cancer vaccines, which are characterized by the use of subunit antigens, has been studied widely in cancer treatment over the years, and many clinical trials have been conducted based on these vaccination strategies. Although the chemistry of antigens is relatively simple, as is the manufacturing and storage process, most cancer vaccine studies have failed to prove clinical benefit in clinical trials. Traditional vaccines are administered by intramuscular injection, where the local immune response is triggered in muscle cells and muscle-resident immune cells before the antigen-presenting cells like DCs infiltrate from circulation to capture antigens [149]. However, the same strategy cannot be employed in the treatment of cancer due to the complex microenvironment of the tumor, especially in advanced tumor models.

For those failed vaccine clinical studies, most of the therapy employed a naked administration of the vaccine, and the rest were compromised by vectors like DCs, viral vectors, and even naked nucleic acids, which can hardly induce immune response because the immune cell evolved as more likely to recognize a dense, highly repetitive epitope arrangement of the antigen. Reports on different vectors in melanoma vaccination have shown the dearth of trial endpoints, and also a clear decrease in commencing since 2008 (**Figure 2**) [150].

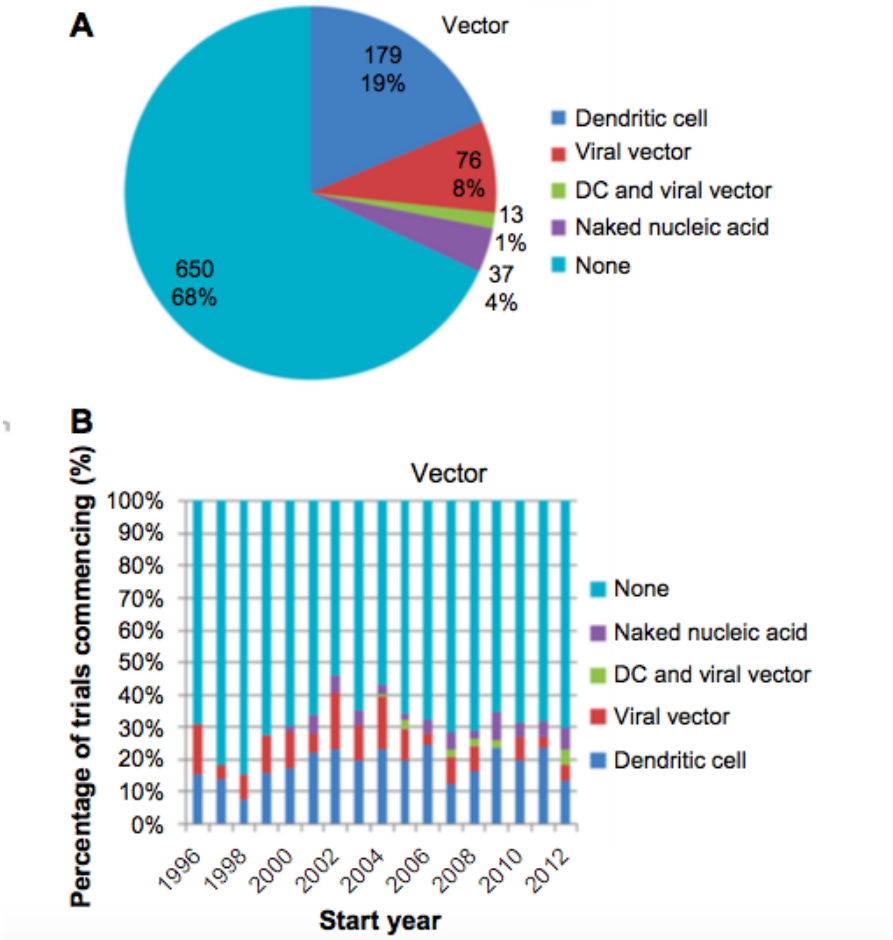



**Figure 2.** Different vectors in melanoma vaccination. (A) Cross-sectional study. (B) Longitudinal study. Reproduced from [150] with permission.

However, the nanoparticle-based vaccine with targeted delivery-loading antigen brought promise to cancer vaccine development [151]. With further understanding of tumor microenvironments, immunotherapy has reshaped the landscape of traditional chemotherapy and made novel delivery target strategies possible [152]. Researchers from Huang's group revealed that the key mechanism of drug resistance in advanced melanoma models is barriers of stroma cells, which are a main component of the tumor immunosuppressive microenvironment. The authors further proved that the suppressive tumor microenvironment could be reversed by silencing the inhibitory cytokines secreted by tumor and stroma cells [153]. By co-formulating the immune-modulating agents with traditional chemotherapy drugs, a synergistic tumor inhibition effect was observed [154, 155]. Additional studies indicated that co-delivering vaccines with microenvironment modulation would greatly enhance treatment of melanoma.

Tumor-associated antigens are vital parts of cancer vaccines that can be used to activate DCs to become antigen-presenting cells (APCs) to present antigen to T cells and kill tumor cells [156]. The recent progress on development of a melanoma vaccine has been made on a nanomedicine platform, which enabled the delivery of endogenous antigens to the cytosol of DCs to induce a cytotoxic T-lymphocyte response [157, 158]. Systemic DC-targeted RNA vaccines formulated with lipid carriers in Phase 1 trials have indicated the possibility of a universally applicable strategy that could formulate polypeptide-antigen-based vaccines in the form of RNA [159]. Direct in vivo delivery of peptide as an antigen has been proved as well. Huang et al. designed lipid-calcium-phosphate (LCP) NPs as intracellular delivery

vesicles to initiate the uptake of self-antigen by DCs (**Figure 3**). The T cells response to vaccines can be greatly enhanced by the use of adjuvants [160].

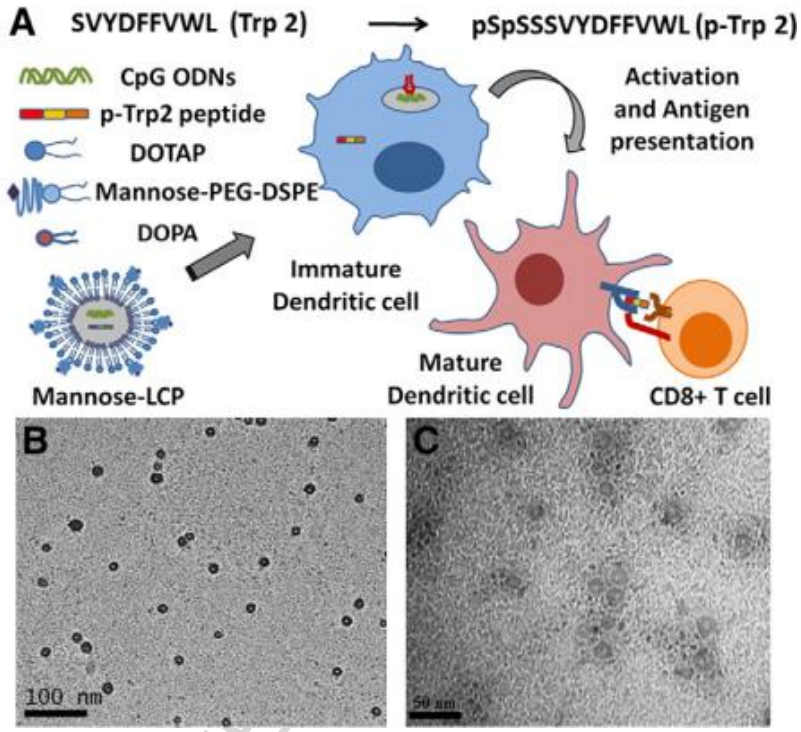



**Figure 3**. LCP vaccine induces potent T cell response. DCs uptake of LCP NPs enhances T cells recruitment (A). TEM images showing NP cores (B) and final structure (C). Reproduced from [160] with permission.

Aluminum salts, which act by generating depots that trap antigens at the injection site, have been used in human vaccines for almost 80 years. In recent years, novel adjuvants, especially pattern recognition receptors (PRR) ligands, have raised much attention. Because PRR activation stimulates the production of cytokines/chemokines that could further increase the host's ability to eliminate the pathogen, the encapsulation of pathogen-associated molecular patterns (PAMPs) in vaccine formulation is expected to enhance and accelerate the induction of vaccine-specific immune response. Adjuvant acts like PAMPs can thus trigger the innate response that threatens the existence of vaccine components with subsequent activation and maturation of APCs that are essential for adaptive immune response [161, 162].

Several studies have shown that co-delivery of immunomodulatory agents, such as PAMP ligands, can enhance the immune response by cancer vaccines. This strategy can be accomplished by nanoparticle-based cancer vaccines, which incorporate the ligands into nanoparticles by encapsulation and covalent conjugation. Several formulation strategies have been developed for ligand conjugation. Adjuvants were co-encapsulated and further modifications were made to enhance the deposition of LNs to initiate stimulation. Superior tumor inhibition was observed in subcutaneous models and metastasis models. Because a considerable number of DCs reside in LNs, LNs can be a good target for cancer vaccines. Direct LN-targeting protein NPs show rapid targeting and prolonged retention, providing a new solution for melanoma treatment [163].

The study and clinical application of checkpoint inhibitors in cancer immunotherapy has shown excellent potential. The existence of checkpoint inhibitors, also called cell PD-1, on T cells, is a vital part of the tumor immunosuppressive resistant that mediates the downregulation of T lymphocytes and makes T cells perfect targets for anti-PD-1 therapy development. In clinical settings, the antibody is given systemically

and significantly increases the survival rate in advanced melanoma patients. Recent reports indicate that the side effects of autoimmune disease were observed due to the blockade of the normal function of T cells residing in normal tissue [164]. Developing a local macromolecule delivery vesicle has been proposed as a solution.

## 4.2 Delivery route and formulation design

### 4.2.1 Systemic route

#### A. Lipoplex

Delivering macromolecule drugs such as siRNA, plasmid DNA (pDNA), and peptide into the basal epidermis area is difficult due to their low permeability through the stratum cornea and epidermis. Thus, the drug has trouble reaching the melanocytes that usually hide in the basal epidermis and upper epidermis. Lipid-based systems were developed for siRNA and achieved different degrees of success. Geusens et al. developed ultradeformable liposomes that could deposit siRNA at the upper dermis in vitro [165]. Dorrani et al. developed a DOTAP-based liposome-siRNA (lipoplex) to enhance penetration and deposition of a BRAF-siRNA that targeted melanoma cells [166]. Other in vivo studies have been performed on lipid-based systems. Lipid-polycation-DNA complex (LPD) was prepared and modified with Polyethylene glycol (PEG), which increased the delivery efficiency fourfold [167-169]. The LCP-based nanoplatform using the microemulsion method to formulate a calcium phosphate (CaP) core coated with cationic lipid and modified with PEG and anisamide ligand was invented to overcome the relatively lower transfection efficiency of siRNA in vivo delivery. LCP has efficiently inhibited lung metastasis in a metastasis melanoma model [170]. Later investigations showed that LCP can also deliver peptide efficiently to DCs, which would be valuable in cancer vaccine development. Further investigation has been conducted by the same group for combined therapy. Trp2 peptide and CpG ODN were co-formulated with LCP, resulting in superior tumor inhibition in B16F10 melanoma models [160]. The problem of the immunosuppressive tumor microenvironment, which had hampered cancer vaccine efficiency in advanced melanoma models, was solved by another lipid-based delivery system. Liposome-protamine-hyaluronic acid (LPH) was found to modulate a tumor immunosuppressive microenvironment characterized by cytokines like TGF-β in an advanced melanoma model treated with cancer vaccine by encapsulated siRNA [171].

#### B. Polymeric NPs

The unmet clinical needs of melanoma treatment can be addressed by the introduction of polymer-based nanomedicine delivery systems. Because most of the anticancer drugs that have been developed for melanoma are lipophilic drugs, their anticancer efficacy is limited due to their unfavorable pharmacokinetic and pharmacodynamic profiles. The introduction of amphiphilic polymers to formulate anticancer drugs successfully altered the delivery profile of the free chemicals. Polymers with biodegradable properties like PEG-b-PLGA are being extensively studied for their hydrophilic properties, which make them attractive for use as anticancer drugs. By applying different formulation strategies, researchers have been able to achieve the enhanced accumulation and retention of NPs, but the issue of the low cellular uptake of NPs by melanocytes is still unaddressed [172-175]. Multiple active targeting strategies are proposed based on the receptors that are overexpressed on melanocytes. Extensive studies have shown that NPs modified with melanoma-related ligands could increase the accumulation amounts significantly [137, 176-178].

Recently, a combination therapy was established on the nanomedicine platform. Multimodality NPs were prepared for the combination of traditional chemotherapy with photodynamic therapy (PDT). Based on the excitation of a photosensitizer at certain wavelengths, PDT is especially useful for detection and treatment of melanoma of various stages, especially metastasized melanoma, and for diagnosis and

destruction of cancer stem cells. Pluronic-based formulations enhance PDT efficacy and overcome the resistance of melanoma cells to PDT [179]. With the utilization of the acid environment of the tumor cells, siRNA and a photosensitizer are formulated with acid-activated cationic micelle for PDT-induced cancer immunotherapy [180]. Together with immunotherapy, which follows local injection of immunotherapeutic agents such as adjuvants, phototherapy has indicated significant clinical value in treating metastasized melanoma.

On the other hand, the application of PDT addresses the inefficiency of NPs caused by the enhanced permeability and retention (EPR) effect. PDT can be used to reduce the high interstitial pressure and increase the vessel permeability, thus facilitating the delivery of NPs. It is reported that the delivery efficiency of liposomal Doxorubicin (DOX) could increase by 75% when combined with PDT [181]. In conclusion, PDT has been shown to be a valuable biomedical tool in the therapy and diagnosis of melanoma.

**C. Nanogel**

Nanogels are NPs that are composed of hydrogels made from cross-linked synthetic polymers. The ability to load small molecules as well as macromolecules facilitates the delivery of multiple therapeutic agents. Various properties can be given to nanogels based on the multimodality of the polymers, making combination therapy for melanoma possible [182].

External near-infrared (NIR) phototherapy can be combined with chemotherapy through the integration of thermosensitive hydrogels with NPs. Nanostructured metal NPs have been designed for phototherapy and cancer cell imaging in recent years. Synergistic therapeutic effects can be achieved by the conjugation of chemotherapy agents with metal NPs for photo-thermally controlled drug release. A nuclear-shell structure of nano-gelatin was prepared by combining the targeted cancer cell, optics temperature, and fluorescence imaging with the chemical photo-thermal method. As a shell, the thermosensitive PEG can be coated to prepare Ag-Au duplex metal NP cores. The combination of chemotherapy and external NIR light therapy can have an interactive effect [183]. Chitosan-based hydrogels can be used for pH-sensitive intracellular delivery. PH-sensitive bio-sensing and imaging can be achieved by immobilization of CdSe quantum dots in chitosan-poly networks [184]. The same group further prepared magnetic-field-sensitive NPs for pH-sensitive release, in which formulated fluorescent carbon dots and super-magnetic iron oxide nanocrystals were prepared as cores coated with hydrogels [185]. The delivery system can manipulate the fluorescence intensity to vary the environmental temperature as well as the promoted release profile of chemotherapy with NIR irradiation and an alternating magnetic field. Fluorescent carbon NPs immobilized within hydrogels showed a similar multifunction therapeutic effect [186].

Apart from traditional chemotherapy, the delivery of proteins by nanogels has been shown to be an alternative method for cancer vaccine delivery. It has been shown that cationic dextran nanogels reversely conjugated with ovalbumin (OVA) with disulfide bonds enable redox-sensitive intracellular release in DCs. Significant therapeutic efficacy was observed when combined with poly (I:C) as the adjuvant [187].

### 4.2.2 Transdermal route

The systemic delivery route has always brought in toxicity concerns on the non-target healthy cells. To better avoiding unsafe side effects, many transdermal delivery systems has been proposed and shown to be a promising alternative for skin-affiliated cancer, especially for the treatment of melanoma. Due to the complex structure of human skin, the challenge for transdermal delivery is to the effective delivery of reagent directly to tumor cells, where skin barriers may greatly compromise the expected therapeutic effect. Among traditional transdermal delivery systems, chemical enhancers were usually applied to increase the penetration of small chemicals, thus showing minimal effect on macromolecule therapy.

Instead, electroporation and microneedles have made much progress in overcoming skin barriers for multi-task delivery. Furthermore, with the help of NP platform, improved efficacy has been achieved.

**A. Electroporation**

The skin permeability of small chemicals as well as macro-molecules can be promoted with electroporation. Due to the poor transfection efficiency and expression of pDNA injection, non-viral gene delivery is enhanced by electroporation or a gene gun. The accessibility and immunogenicity of skin make it the perfect site for electroporation, though the low permeability of the stratum cornea hinders the delivery of macro-molecules like pDNA and protein.

Cytokine delivery is becoming a promising strategy for melanoma therapy that can boost the immune response to tumor antigens. However, the delivery of commercially available cytokines as recombinant proteins may bring some side-effect issues. Electroporation of pDNA against melanoma was studied, and great progress has been made so far. In vivo electroporation of pDNA has been extensively performed in melanoma models. For instance, interleukin-12 (IL-12) electroporation could boost systemic immune responses that kill tumor cells in a specific manner [188]. Similarly, tumor regression was observed after interleukin-15 (IL-15) electroporation in a melanoma model. It was shown that electroporation is a potential delivery approach that can be used to avoid systemic intravenous cytokine delivery [189]. Several clinical studies have applied electroporation for DNA vaccines and have shown it to be a promising method of cancer vaccination [190, 191].

**B. Microneedles**

In recent years, more and more studies have focused on transdermal delivery by microneedles. Microneedles hold promise as a minimally invasive delivery route that may promote delivery efficiency by bypassing the stratum cornea [192]. Microneedles prepared by biodegradable materials could dissolve in skin layers quite quickly and release the preloaded agents into skin tissue [193, 194]. Studies of microneedles range from traditional small-molecule drugs to novel macromolecule delivery to melanomas, and significant progress has been made.

The anti-tumor effect of traditional, commercially available 5-FU topical cream is greatly enhanced when applied to skin pretreated with microneedles [195]. Due to the poor permeability of the skin, it is difficult to deliver macromolecules and NPs by the transdermal route. By physically piercing micro-sized pores in the stratum cornea layer in the skin, microneedles facilitate the transdermal delivery of novel macromolecule-based therapies. Research shows that a microneedle array could facilitate the DCs' uptake of antigens, which may increase the vaccine's immunogenicity. Robust, antigen-specific immune response was observed after the application of microneedles laden with antigen-encapsulated PLGA NPs [196].

Studies have indicated that the application of anti PD-1 antibodies has significant clinical value, while more and more reports show that excess of antibodies in patients caused by systemic delivery would increase the risk of side-effect autoimmune disease. Additionally, systemic delivery of antibodies could hardly enhance the local concentration of antibodies in tumors. Although electroporation delivery of pDNA has been shown to be effective for melanoma in clinical studies, the transient pain is the major drawback in clinical settings.

Clinical needs have led researchers to design smart microneedle delivery techniques for antibody against PD-1 (the aPD-1, see **Figure 4**) [197, 198]. Since microneedles could not respond to the tumor environment with adjusted release profiles, researchers developed dual-response microneedles. The retention of antibodies in the tumor environment was increased and enzyme-mediated sustained drug release was created by integrating the microneedles with pH-sensitive dextran NPs.

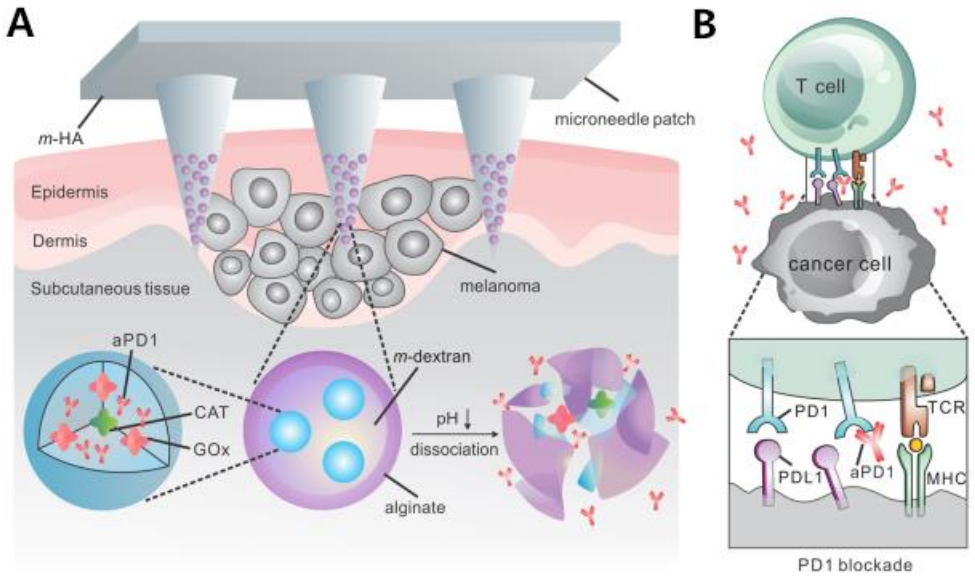



**Figure 4**. Microneedle delivery of aPD-1 induces in-situ immune activation. (a) Schematic depicting aPD-1 delivery. (b) PD-1 blockade by aPD-1. Reproduced from [197] with permission.

These microneedles are prepared with biocompatible hyaluronic acid (HA) and integrated with antibodies and glucose oxidase, which converts blood glucose into acid that promotes the self-degradation of NPs. The sustained release of antibodies into the tumor environment has been shown to be more effective than systemic delivery of antibodies and intratumor delivery of NPs. Although intratumor delivery of aPD-1 NPs would increase the average survival rate, it is far less effective compared to microneedle delivery. Also, the risk of autoimmune disease after exposure to high concentrations of antibodies delivered systemically was reduced due to the relatively low dosage of antibodies with microneedles [199]. The research also shows that there is a possibility of combining NPs with microneedles in melanoma treatments.

### C. Nanoparticles

The ability of chemical reagents to penetrate through human skin would be largely improved when encapsulated into NP formulations. Typical NPs used in transdermal delivery includes traditional liposomes, solid lipid NPs and polymer-based NPs. Beside traditional anti-cancer formulations such as DOXIL® - the Doxorubicin liposomal, PDT therapy applied on liposomal deliveries would largely increase the penetration strength by several folds. On the other hand, polymer-based NPs, compared with liposomes, tend to actively interact with corneocyte in a way that also improve the sustained release of anti-cancer drugs.

Many nanoplatforms have been well designed to increase the penetration of poorly soluble drugs. For instance, it is reported that 5-FU or curcumin formulated in nanogels have shown promising benefits for topical delivery [200]. Furthermore, topical delivery of macromolecules can be greatly facilitated by nanotechnology. Recently, carbon nanotubes based siRNA delivery system has been reported in successfully delivering of siRNA against BRAF, thus to inhibit aggressive tumor progression [201].

In conclusion, NPs would enhance overall therapeutic benefits by facilitating the penetration of therapeutic agents across human skin barriers, as well as reducing side effect caused by traditional systemic route.

## Summary and future perspective

Management of advanced melanoma is still a major challenge, and gaining a better understanding of melanoma biology is essential to address the challenges associated with existing therapies. To effectively diagnose and treat melanoma, and also provide preventive insights, we learn from successful animal models that assemble relative clinical settings. Adoptive T-cell therapy, therapeutic vaccines, and chimeric antigen receptor (CAR) T-cell therapy are some of the novel strategies currently being explored in clinical trials. NPs can be potentially exploited as efficient drug delivery vehicles and may reduce side effects associated with some of the present therapeutics. Theoretically, the NP platforms can be exploited for combinatorial therapy by designing multimodal particles. However, clinical translation of NP technologies is currently far from optimal.

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