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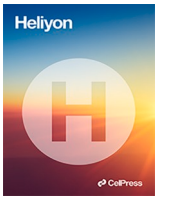

Review article

Gold nanostructures in melanoma: Advances in treatment, diagnosis, and theranostic applications

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Abbreviations: 2-NNA, 2-Naphthaleneacetic Acid; 5-FU, Fluorouracil; ADA, Adamantane; AI, Artificial Intelligence; APC, Antigen-Presenting Cells; Apt, Aptamer; BAK, Bcl-2 homologous antagonist/killer; BRAF, V-Raf Murine Sarcoma Viral Oncogene Homolog B; BSA, Bovine Serum Albumin; β-CD, β-Cyclodextrin; CD, Cluster of Differentiation; CGH, Comprehensive Genomic Hybridization; CpG, Cytosine-Guanosine Dinucleotide; CTL, Cytotoxic T Lymphocyte; CT, Computed Tomography; CTAB, Cetyltrimethylammonium Bromide; Cy5, Cyanine 5; Cy5-DEVD, Cy5-Tagged Caspase-3 Specific Peptides; DC, Dendritic Cell; DOX, Doxorubicin; DR5, Death Receptor 5; DLS, Dynamic Light Scattering; ER, Endoplasmic Reticulum; EPR, Enhanced Permeation and Retention; E. coli, Escherichia coli; FDA, Food and Drug Administration; FAK, Focal Adhesion Kinase; FLI, Fluorescence Imaging; FRET, Fluorescence Resonance Energy Transfer; FTIR, Fourier-Transform Infrared Spectroscopy; GEM, Genetically Engineered Mice; GD2, Ganglioside D2; GFP, Green Fluorescent Protein; GNP, Gold Nanoparticle; GNC, Gold Nanocage; GNC, Gold Nanocluster; GNR, Gold nanorod; GNS, Gold Nanoshell; HA, Hyaluronic Acid; HER2, Human Epidermal Growth Factor Receptor 2; HLA, Human Leukocyte Antigen; HGN, Hollow Gold Nanoparticles; IgG, Immunoglobulin G; IKKβ, Inhibitor of Nuclear Factor Kappa-B Kinase Subunit Beta; IL, Interleukin; ISQ, Squaraine; LbL, Layer-by-Layer; LED, Light-Emitting Diode; LPS, Lipopolysaccharides; MAPK, Mitogen-Activated Protein Kinases; MAGE-1, Melanoma-Associated Antigen 1; MC1R, Melanocortin 1 Receptor; MART-1, Melanoma-Associated Antigen Recognized by T Cells 1; MEK, Mitogen-Activated Protein Kinase; MIA, Melanoma Inhibitory Activity; ML, Machine Learning; MPC, Monolayer-Protected Clusters; MRI, Magnetic Resonance Imaging; MPLA, Monophosphoryl Lipid A; NF-κB, Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; NK cells, Natural Killer Cells; NIR, Near-Infrared; NDP-MSH, [Nle4, D-Phe7]-α-Melanocyte-Stimulating Hormone; OVA, Ovalbumin; ODV, Optical Droplet Vaporization; OMV, Outer Membrane Vesicle; PA, Photoacoustic Imaging; PAMP, Pathogen-Associated Molecular Patterns; *Pc*, Phthalocyanine; PCR, Polymerase Chain Reaction; PD1, Programmed Cell Death Protein-1; PEG, Polyethylene Glycol; PET, Positron Emission Tomography; PET/CT, Positron Emission Tomography/Computed Tomography; PFH, Perfluorinated Hexane; PI3K/AKT, Phosphatidylinositol 3-Kinase/Protein Kinase B; PDT, Photodynamic Therapy; PDX, Patient-Derived Xenograft; pDNA, Plasmid DNA; PS, Photosensitizing Agent; PTX, Paclitaxel; ROS, Reactive Oxygen Species; RGD, Arginine-Glycine-Aspartic Acid; scFv, Single-Chain Variable Fragment; SDT, Sonodynamic therapy; SELEX, Systematic Evolution of Ligands by Exponential Enrichment; SEM, Scanning Electron Microscopy; SERS, Surface-Enhanced Raman Spectroscopy; siRNA, Small Interfering RNA; SPR, Surface Plasmon Resonance; STAT 3, Signal Transducer and Activator of Transcription 3; SWCNT, Single-Wall Carbon Nanotubes; SYMPHONY, Synergistic Immuno Photothermal Nanotherapy; TCR, T-Cell Receptor; TGNP, Titania/Gold nanoparticle; TIL, Tumor Infiltrating Lymphocyte; TLR, Toll-Like Receptors; TRP2, Tyrosinase-Related Protein 2; UV, Ultraviolet; UW, Ultrasound Waves; XPS, X-Ray Photoelectron Spectroscopy.

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ABSTRACT

Melanoma, a lethal form of skin cancer, poses a significant challenge in oncology due to its aggressive nature and high mortality rates. Gold nanostructures, including gold nanoparticles (GNPs), offer myriad opportunities in melanoma therapy and imaging due to their facile synthesis and functionalization, robust stability, tunable physicochemical and optical properties, and biocompatibility. This review explores the emerging role of gold nanostructures and their composites in revolutionizing melanoma treatment paradigms, bridging the gap between nanotechnology and clinical oncology, and offering insights for researchers, clinicians, and stakeholders. It begins by elucidating the potential of nanotechnology-driven approaches in cancer therapy, highlighting the unique physicochemical properties and versatility of GNPs in biomedical applications. Various therapeutic modalities, including photothermal therapy, photodynamic therapy, targeted drug delivery, gene delivery, and nanovaccines, are discussed in detail, along with insights from ongoing clinical trials. In addition, the utility of GNPs in melanoma imaging and theranostics is explored, showcasing their potential in diagnosis, treatment monitoring, and personalized medicine. Furthermore, safety considerations and potential toxicities associated with GNPs are addressed, underscoring the importance of comprehensive risk assessment in clinical translation. Finally, the review concludes by discussing current challenges and future directions, emphasizing the need for innovative strategies to maximize the clinical impact of GNPs in melanoma therapy.

1. Introduction

Melanoma, recognized as the deadliest type of skin cancer, arises from the uncontrolled proliferation of melanocytes and is responsible for more than 80 % of skin cancer-related fatalities [1]. According to WHO (www.iarc.who.int) report in 2022, approximately 330,000 new melanoma cases were diagnosed worldwide, and about 60,000 people died from the disease. In the United States, advanced melanoma patients typically survive 3–11 months, with a five-year survival rate of less than 10 % for metastatic cases. However, the five-year survival rates are 99.4 % for stage I and II, 68.0 % for stage III, and 29.4 % for stage IV melanoma patients [2]. Melanoma development is influenced by genetic predisposition, hereditary factors, and environmental influences, with ultraviolet (UV) radiation being the primary risk factor [3,4]. Melanoma can be categorized into cutaneous and non-cutaneous subtypes.

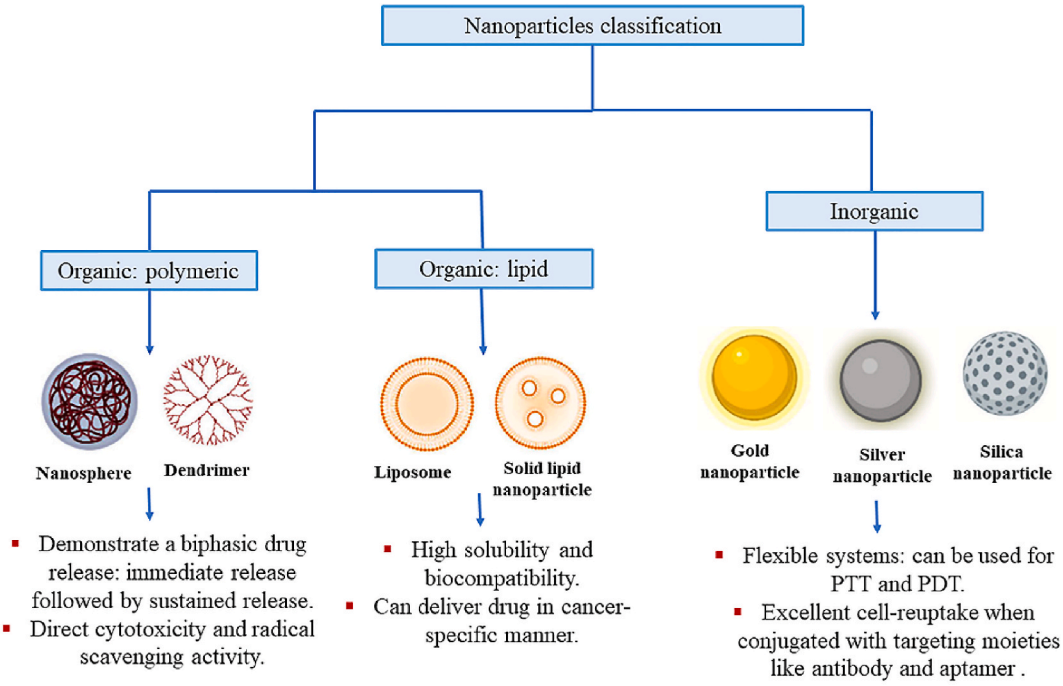

Fig. 1. Nanoparticle types applicable in skin cancers: a) polymeric nanoparticles offer enhanced bioavailability and a controlled release profile, but they are limited by complex manufacturing and potential toxicity; b) lipid nanoparticles offer high biocompatibility and biodegradability, but reduced payload capacities and stability challenges limit them, c) inorganic nanoparticles provide uniquely tunable sizes, shapes, and conjugations, but concerns regarding biodegradability and long-term toxicity limit them [20]. PTT: photothermal therapy; PDT: photodynamic therapy.

Cutaneous melanoma constitutes over 90 % of melanoma cases and is associated with UV exposure. In contrast, non-cutaneous melanoma, which appears in areas without skin, such as acral, uveal, and mucosal regions, is less influenced by UV exposure, with mucosal melanoma being the rarest form [5]. A study examining 428 patients with metastatic melanoma treated with checkpoint inhibitors between 2007 and 2018 revealed 5-year survival rates of 46 % for cutaneous melanoma, 34 % for acral melanoma, 21 % for uveal melanoma, and 22 % for mucosal melanoma [6]. Understanding the molecular mechanisms of cancer is crucial for developing effective, tailored therapies to improve patient outcomes [7]. The typical molecular pathways that experience irregularities in melanoma include mitogen-activated protein kinases (MAPK) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), along with mutations in the general cyclin-dependent kinase inhibitor 2A (CDKN2A) [5]. The V600 BRAF mutation, present in 50 % of melanoma cases, activates the MAPK pathway [8]. Early detection of melanoma is crucial, but diagnosis often relies on biopsies and immunohistochemistry, leading to potential misdiagnoses as melanoma can mimic benign lesions [9]. Surgical removal is the primary treatment but is not curative due to melanoma's high metastatic potential. Immune checkpoint inhibitors have improved survival rates but face challenges of non-response, resistance, and severe side effects, with chemotherapy as a secondary option [9]. Tumor-infiltrating lymphocyte (TIL) and T-cell receptor (TCR)-based therapies show promise but also have limitations [5]. Non-cutaneous melanomas respond poorly to existing treatments, highlighting the need for novel therapies [8]. Overall, there are challenges in melanoma diagnosis and treatment, including variable responses, resistance, and the need for more precise methods.

Nanotechnology involves novel materials, tools, and systems by exerting control at the molecular and atomic levels and harnessing their properties at the nanoscale [10–12]. One of the extensively scrutinized domains of medical nanotechnology pertains to the diagnosis and treatment of melanoma. Nanoparticles enhance drug delivery by targeting cancer cells, improving therapeutic efficacy, and reducing side effects [13,14]. Nanotechnology also plays a significant role in diagnostics, with nanoparticles used to create sensitive and specific imaging agents [15]. Various nanoparticle platforms, such as liposomes and niosomes, polymer-based nanoparticles, carbon nanotubes, dendrimers, cubosomes, and noble metal nanoparticles (such as silver and gold), have been developed for the diagnosis and treatment of melanoma (Fig. 1) [16–18], with liposomes and polymeric nanoparticles advancing to clinical trials [19].

Gold nanostructures, such as gold nanoparticles (GNPs), characterized by their unique optical and physicochemical properties, hold immense promise in diagnostics, therapeutics, and theranostics (Fig. 2). These nanoparticles, often tunable in size and shape, can be functionalized for biomedical applications. In therapeutics, gold nanostructures are employed as drug carriers, allowing targeted delivery to disease sites and reducing off-target effects. Unlike polymeric and lipid nanoparticles, GNPs have higher skin penetration efficiency due to their small sizes, making them a viable option for transdermal delivery [21]. In addition, GNP's ability to absorb and convert near-infrared light into heat makes them ideal candidates for photothermal therapy (PTT) and photodynamic therapy (PDT). In diagnostics, gold nanostructures offer sensitive and specific platforms for detecting biomolecules, making them invaluable in colorimetric, conductive, or electrochemical assays for various diseases, including cancer markers. GNPs demonstrate remarkable stability and are resistant to photobleaching, rendering them an excellent option for use as contrast agents in bioimaging applications [22]. In addition, their strong light-scattering properties make them excellent contrast agents for imaging techniques such as photoacoustic, computed tomography (CT), and surface-enhanced Raman spectroscopy (SERS), enabling precise disease visualization. Early cancer diagnosis is essential for effective treatment. GNPs, with their biocompatibility and strong optical scattering behavior,

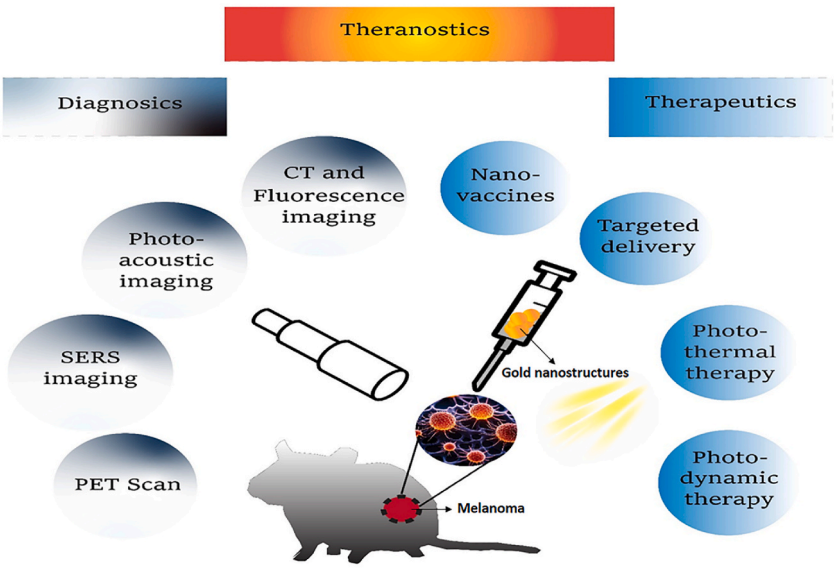

Fig. 2. A general scheme summarizing the applications of gold nanostructures in melanoma. SERS: Surface-Enhanced Raman Spectroscopy, PET: Positron Emission Tomography, CT: Computed Tomography. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

serve as ideal probes for cancer imaging. Conjugated antibodies enable GNPs to target malignant cells, especially those overexpressing specific markers, allowing precise localization within the body. Targeting markers like epidermal growth factor receptors demonstrates GNPs' potential for imaging applications in both laboratory models and live subjects [23]. Combining diagnostic and therapeutic functions, gold nanostructures find their niche in theranostics. By integrating imaging and therapeutic modalities, they enable personalized medicine approaches, facilitating real-time monitoring of treatment response. These versatile nanoparticles represent a promising frontier in modern medicine, offering multifaceted solutions for improved patient care and outcomes [24,25].

This review provides the latest advances in harnessing diverse gold nanostructures for their pivotal roles as diagnostic, therapeutic, or theranostic agents for melanoma management. This topic has not been comprehensively addressed since 2018 [26]. Unlike previous reviews on GNPs [27] or melanoma [26,28], this review offers a thorough exploration of therapeutic, diagnostic, and theranostic aspects, ensuring that the importance and intricacies of GNPs in melanoma management receive adequate attention. Special emphasis is placed on enhancing the understandability and accessibility of the content, achieved through the inclusion of figures, tables, and additional explanations to elucidate complex concepts and novel approaches such as vaccines and theranostics. By offering novel insights and perspectives, this review contributes to advancing the field of GNPs for melanoma research and clinical practice.

Throughout the review, we discuss the multifaceted applications of gold nanostructures in melanoma care, shedding light on their use as diagnostic tools capable of sensitive and specific detecting melanoma biomarkers and imaging. Radioactive gold nanostructures can also be synthesized by employing the ^{198}Au isotope as the precursor material, which holds potential applications in multimodality imaging techniques as well as cancer therapy [29–31]. Moreover, we delve into their therapeutic potentials, elucidating how these nanostructures serve as drug carriers, enabling precise and targeted delivery to melanoma sites while minimizing damage to normal tissue. Their exceptional ability to convert near-infrared light into localized heat, paving the way for photothermal therapy, is also examined. Furthermore, this review underscores the paramount importance of theranostics in melanoma management, emphasizing the integration of diagnostic and therapeutic functionalities enabled by gold nanostructures. This powerful combination allows for real-time tracking of treatment response and the development of personalized therapeutic regimens. Finally, we conclude and offer insights into the prospects of utilizing gold nanostructures in melanoma management.

2. Melanoma: diagnosis and treatment

Early detection of melanoma is vital due to its life-threatening nature. Suspected atypical lesions are biopsied, with diagnosis primarily relying on immunohistochemistry, despite ongoing development of advanced methods like gas chromatography/mass spectrometry and electrochemical detection [32,33]. However, melanoma can mimic benign skin lesions, leading to potential misdiagnoses [32]; Hence, improving diagnostic accuracy beyond conventional methods is essential. Common melanocytic markers, such as melanoma-associated antigens recognized by T cells (MART-1), often fail to distinguish melanoma from benign proliferation [9]. Novel diagnostic approaches, including comprehensive genomic hybridization (CGH) [34], fluorescence in situ hybridization (FISH) [35], and gene expression profiling (GEP)-based analyses [36,37], are being explored to differentiate melanoma from benign or low-grade dysplastic nevi. Systems biology and omics technologies, utilizing automated DNA sequencers, microarrays, and mass spectrometry, provide comprehensive insights into diseases and therapies [38]. Recent advancements in omics technologies promise to identify clinically relevant biomarkers for inflammation, aiding in early diagnosis, prevention, or interception of melanoma. These biomarkers can also predict personalized therapeutic responses and patient survival [39].

The primary treatment for melanoma is surgical removal of the tumor along with a wide margin of healthy tissue. Still, due to the high metastatic potential, this approach is not curative [9]. Extensive research has focused on the dysfunctional immune responses in cancer patients [40]. The most effective treatments for metastatic melanoma are immune checkpoint inhibitors, which have significantly improved survival rates by countering the mechanisms by which melanoma cells evade the immune system. However, many patients either do not respond, develop resistance or cannot tolerate the severe side effects [9]. Chemotherapy, specifically dacarbazine, remains a secondary option when targeted therapies and immunotherapies fail or are unavailable [9]. Current clinical trials for cutaneous melanoma focus on immunotherapy, targeted therapy, and their combinations, with or without chemotherapy [8]. Pharmacotherapy of cutaneous melanoma is summarized in Table 1. A notable phase III trial, IMspire150, is testing a combination of atezolizumab, vemurafenib, and cobimetinib for BRAF-positive advanced melanoma, but has been linked to severe adverse events such as fulminant hepatitis and hepatic failure [8,41]. Over a hundred trials are exploring TIL therapy, which shows promise for end-stage patients but can cause severe side effects when combined with interleukin-2 (IL-2) and chemotherapy. In 2022, a phase I trial began for a TIL therapy featuring membrane-bound IL-15, eliminating the need for additional IL-2 administration [5]. Non-cutaneous melanomas, particularly mucosal and uveal types, respond poorly to immune checkpoint inhibitors, highlighting the need for novel therapies [8]. Various dendritic cell (DC) vaccines are in clinical trials for cutaneous and non-cutaneous melanomas [42]. An ongoing phase I trial for uveal melanoma involves an RNA-transfected DC vaccine. This vaccine holds a notable advantage, incorporating a stabilized mutant of inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ) that activates the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. This activation enhances co-stimulatory molecule expression, improving memory cytotoxic T lymphocyte (CTL) responses and natural killer (NK) cell activation, thus increasing the vaccine's effectiveness and personalization [42]. TCR-based adoptive therapy involves the infusion of genetically modified T-lymphocytes expressing TCR. Currently, there are over 70 ongoing clinical trials based on TCR therapy [5]. Tebentafusp, FDA-approved in 2022, targets the melanoma antigen gp100 and activates $CD3^+$ T cells through a TCR connected to an anti-CD3 single-chain variable fragment [43,44]. The TCR is connected to a single-chain variable fragment targeting anti-CD3, activating $CD3^+$ T cells. However, Tebentafusp requires the presence of the HLA-A*02 allele in patients, excluding many patients due to global HLA-A*02 subtype diversity [44].

Table 1
Primary cutaneous melanoma pharmacotherapy in 2024 (adapted from Dixon et al. J. Clin. Med. 2024, 13(6), 1607; DOI: https://doi.org/10.3390/jcm13061607. Licensed under a Creative Commons Attribution 4.0 International License (CC BY).).

Drug Combination	Class	Indication in Trial	Melanoma-specific survival Established Benefit	Adverse Events	Reference No.
Dabrafenib and Trametinib	BRAF inhibitor + MEK inhibitor	Unresectable stage 3 or 4 melanoma	72 % 12-month survival, 44 % 3-year survival, 28 % 5-year survival	Gastrointestinal adverse events, pyrexia (fever), and peripheral edema	[45–48]
		Involvement of lymph nodes following complete resection	58 % 3-year relapse-free survival, 86 % 3-year overall survival		
Dabrafenib alone	BRAF inhibitor	Unresectable stage 3 or 4 melanoma	32 % 3-year survival, 5.1-month median progression-free survival	Fatigue, alopecia, photosensitivity, nausea, diarrhea, arthralgia (severe, leading to therapy cessation)	[45,47,49,50]
Vemurafenib alone	BRAF inhibitor	Unresectable stage 3 or 4 melanoma	65 % 12-month survival, 17-month median overall survival, 15.9-month median overall survival, 7.3-month median progression-free survival, 17 % 4-year survival	Fatigue, alopecia, photosensitivity, nausea, diarrhea, arthralgia (severe, leading to therapy cessation)	[45,47,51,52]
Vemurafenib and Cobimetinib	BRAF inhibitor + MEK inhibitor	Unresectable or metastatic melanoma	22.3-month median overall survival, 9.9-month median progression-free survival	Rash, diarrhea, photosensitivity, elevated creatine phosphokinase, serous retinopathy, pyrexia, and liver laboratory abnormalities	[45,51–53]
Encorafenib and Binimetinib	BRAF inhibitor + MEK inhibitor	Unresectable or metastatic melanoma	14.9 months median progression-free survival	Increased gamma-glutamyl transferase, hypertension, myalgia, and arthralgia	[45,54,55]
Pembrolizumab	PD1 monotherapy	Unresectable or metastatic melanoma	55 % 2-year survival	Hypothyroidism (14 % of patients), other endocrine disorders, grade 3 to 4 adverse events in 15 % of patients, chronic immune-related adverse events	[45,56]
		Lymph node involvement following complete node dissection	75 % 12-month recurrence-free		
Nivolumab	PD1 monotherapy	Unresectable or metastatic melanoma	52 % three-year survival, 6.9-month median progression-free survival	Grade 3 or greater adverse events like pembrolizumab and chronic immune-related adverse events with potential persistence	[45,57,58]
		Lymph node involvement or metastatic disease following complete resection of lymph node involvement or complete resection of metastatic disease	70 % 12-month recurrence-free survival		
Ipilimumab alone	CTL-4 monotherapy	Unresectable or metastatic melanoma	61 % recurrence-free at 12 months, 34 % survival at 3 years, 2.9-month median-free survival, 43 % 2-year survival, 65 % 5-year recurrence-free (Stage III disease)	43 % discontinued, 2 deaths, Immune-related colitis, Liver impairment, Diarrhea Dehydration, Fatigue Confusion, Skin rashes	[45,56,59]
Ipilimumab and Nivolumab combination	CTL-4 therapy + PD1 therapy	Unresectable or metastatic melanoma	58 % survival at 3 years, 11.5-month median progression-free survival, 75 % recurrence-free survival at 12 months	Severe (grade 3) adverse events in 59 % of patients	[45,57,58,60,61]
Tremelimumab alone	CTL-4 therapy	Unresectable stage IIIc or IV melanoma	20.7 % survival at 3 years	Diarrhea, pruritus, rash, endocrine toxicities	[62]

(*continued on next page*)

Table 1 (*continued*)

Drug Combination	Class	Indication in Trial	Melanoma-specific survival Established Benefit	Adverse Events	Reference No.
			Median overall survival: 12.6 months	Serious adverse events (52 %), 7 % grade 5 adverse events	
Tebentafusp Durvalumab Tremelimumab	gp100 peptide-HLA-directed CD3 T cell engager PD-1 therapy CTL-4 therapy	Unresectable or metastatic melanoma	76 % 1-year overall survival, median overall survival of 18.7 months	Manageable cytokine release syndrome, Pruritus, Grade 3 rash, increase in serum cytokines and chemokines and T-cell trafficking from the blood, Generalized edema	[63]
Atezolizumab, Vemurafenib, and Cobimetinib	CTL-4 therapy BRAF inhibitor + MEK inhibitor	Unresectable or metastatic melanoma	Median overall survival: 39.0 months	Blood creatine phosphokinase increased, diarrhea, pyrexia, Serious adverse events (48 %), 3 % grade 5 adverse events	[64]
Imatinib	c-KIT (tyrosine kinase) inhibitor	Unresectable or metastatic melanoma	Median overall survival: 13.1 months, Median progression-free survival: 4.2 months	Edema (50 %), rash (18 %), fatigue (9 %), anorexia (7 %), nausea (5 %), and neutropenia (2 %)	[65]
Lifileucel	Tumor-Infiltrating Lymphocyte (TIL) Therapy	Unresectable or metastatic melanoma previously treated with immune checkpoint inhibitors	58 %1-year overall survival, Median overall survival: 17.4 months	Chills, pyrexia, fatigue, tachycardia, diarrhea, febrile neutropenia, edema, rash hypotension, alopecia, infection, hypoxia, and dyspnea, Grade 3/4 treatment-emergent adverse events (≥30 %), thrombocytopenia (76.9 %), anemia (50.0 %), and febrile neutropenia (41.7 %)	[66,67]

Challenges in melanoma diagnosis and treatment include variable patient responses, resistance to therapies, and the need for precise diagnostic methods. Additionally, there is a lack of optimized, melanoma-specific human models. Genetically engineered mice (GEM) and patient-derived xenograft (PDX) models have limitations, such as low mutation rates and inadequate representation of human immune systems. Organoids and alternative animal models, like zebrafish, are relatively cost-effective for screening and large-scale production [68]. Zebrafish have epithelium-associated melanocytes that migrate to tumor sites, with a 96 % similarity to the human BRAF gene. In addition, the "TEAZ" method (transgene electroporation in adult zebrafish) allows temporary manipulation of melanocytes [68,69]. Despite advancements, these challenges highlight the need for more effective and accurate research models to improve melanoma diagnosis and treatment.

3. Gold nanostructures: synthesis, characterization, and biomedical applications

Gold nanostructures have found widespread use in numerous applications due to their straightforward synthesis, distinctive physicochemical characteristics, robust chemical stability, small size, and ease of functionalization through physical absorption or reactive groups such as amines, thiols, and carboxyl groups. These qualities collectively enhance their remarkable capability for biofunctionalization and loading, making gold nanostructures highly effective for targeting therapeutic or imaging applications [21,70]. GNPs have been utilized for decades and are widely regarded as possessing the greatest potential for biomedical applications compared to other metallic nanoparticles [71]. Among inorganic nanomaterials, silver and gold nanostructures exhibit lower toxicity. However, silver nanomaterials are more cytotoxic than asbestos at specific concentrations [72].

The synthesis of diverse gold nanostructures demands specialized methods for tunning properties and potential applications of each nanostructure. For instance, GNPs are typically generated through chemical reduction methods utilizing agents like sodium citrate or sodium borohydride, enabling the production of spherical particles with adjustable sizes. A seed-mediated growth approach is commonly employed to prepare gold nanorods (GNRs). In this method, small gold seeds initiate the growth of elongated rods when a gold precursor and a cationic surfactant, such as cetyltrimethylammonium bromide (CTAB), are introduced. A similar seed-mediated growth approach is used for the synthesis of gold nanostars, where initial gold seeds are guided to form branched star-shaped structures by adding growth agents and surfactants. The synthesis of gold nanoplates often relies on a seed-mediated growth strategy. By meticulously controlling reaction kinetics, particularly the addition rate of gold ions and the type of capping agents utilized, gold nanoplates of various shapes, such as triangular or hexagonal, can be obtained. Hollow gold nanoparticles (HGNs) such as gold nanocages are typically synthesized through a Galvanic replacement reaction. In this process, silver templates such as nanocubes are transformed into hollow gold nanostructures by immersing them in a gold salt solution. The silver nanoparticle-assisted growth method is commonly used to produce gold nanowires in solution. Silver nanoparticles catalyze the anisotropic growth of gold, yielding wire-like structures. For gold nanoshell synthesis, silica nanospheres serve as templates, and a gold layer is deposited onto their surfaces. Subsequent etching of the silica core results in the formation of hollow gold nanoshells [73,74]. Gold nanoclusters are

fabricated by reducing Au^{3+} ions to Au^{0} using appropriate reducing agents like borohydrides or citrate. The distinctive advantage of gold nanoparticles lies in their ability to be designed flexibly through controlled and easily managed synthesis methods. Additionally, GNPs can be linked with other molecules to enhance overall properties, ranging from biological activity to stability [75]. For instance, hollow gold nanoparticles possess several attributes that make them appealing for applications such as PTT, SERS, and drug delivery. However, they exhibit lower biocompatibility and stability compared to other gold nanostructures. Incorporating a water-soluble stabilizing agent, such as soluble supramolecular calixarene (SC4), can enhance these properties [76].

Characterizing these gold nanostructures is vital to ensure their quality and performance. Techniques like transmission electron microscopy (TEM) (Fig. 3) and scanning electron microscopy (SEM) are instrumental in visualizing the size, morphology, and uniformity of these nanostructures. UV–visible spectroscopy can provide information on their optical properties, while X-ray diffraction (XRD) offers insights into their crystalline structure. SERS is a powerful tool for probing the surface chemistry and functionalization of gold nanostructures, while dynamic light scattering (DLS) can determine their size distribution and stability in solution. Furthermore, techniques such as X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectroscopy (FTIR) can elucidate their chemical composition and surface functional groups, ensuring the successful synthesis of various gold nanostructures tailored for specific applications.

Gold nanostructures demonstrate remarkable versatility with applications spanning diverse fields (see Table 2). Due to their biocompatibility and ease of functionalization, gold nanostructures are utilized for targeted drug, gene, or vaccine delivery. For instance, fluorouracil-loaded gold nanoparticles have been incorporated into gels and creams, resulting in twice the skin permeability and significantly reduced tumor volumes in mice with A431 tumors compared to free 5-FU formulations [83]. In another study, botulin-conjugated GNPs were easily formed by combining a drug solution with citrate-capped GNPs, showing a dose-dependent reduction in cell viability of human melanoma cells [84]. Researchers also harness the capabilities of gold nanostructures, including non-spherical shapes like gold nanocages, to address melanoma. For example, Poudel et al. utilized gold nanocages to create liposome-coated versions loaded with melanoma antigens and adjuvants, enhancing the anti-melanoma immune response by their hollow structure as a reservoir for antigen loading [85]. Furthermore, gold nanostructures prove highly effective in cancer PTT and PDT, capitalizing on their unique optical properties. In medical imaging, these nanostructures enhance CT imaging contrast, enabling more precise diagnostics, while their proficiency in generating SERS signals is leveraged for sensitive molecular imaging. They play a pivotal role in biosensors, significantly advancing diagnostic capabilities. Moreover, gold nanostructures facilitate multimodal imaging techniques, offering complementary insights into complex biological systems through optical coherence tomography,

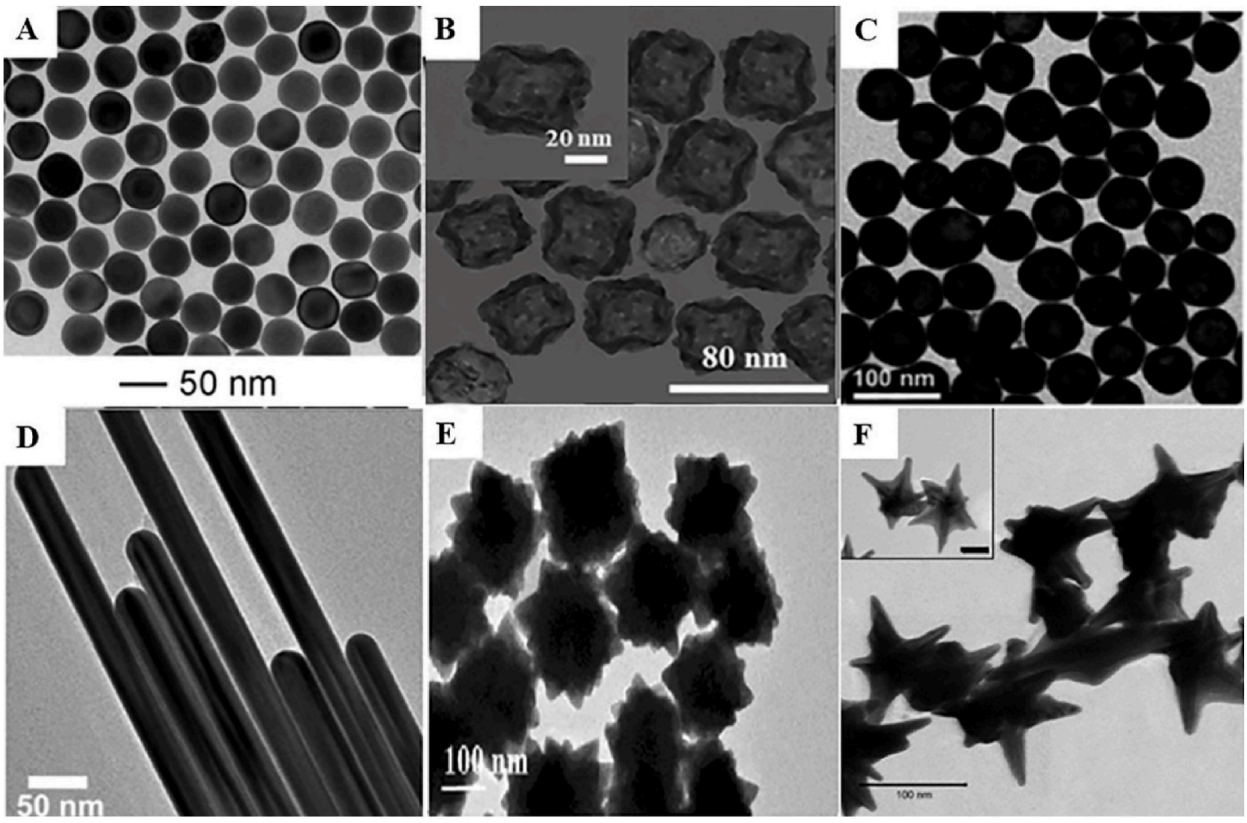

Fig. 3. TEM image of A) Gold nanospheres [77], B) Gold nanocages [78], C) Hollow gold nanospheres [79], D) Gold nanowires [80], E) Gold nanoflowers [81], and F) Gold nanostars [82].

Table 2
Various gold nanostructures and their potential applications.

Gold nanostructure	Synthesis	Size	Applications	Reference
Nanospheres	Chemical reduction method	5–100 nm	Peptide vaccine immunotherapy, PTT, CT, photoacoustic imaging, and SERS.	[73,74,88,89]
Hollow nanospheres	Galvanic replacement	30–60 nm	PTT, drug delivery, photothermal transfection, photoacoustic imaging, SERS.	[73,74,90,91]
Nanorods	Seed-mediated growth	40–100 nm	Photoacoustic/ultrasound dual-mode imaging, SERS, two-photon luminescence imaging, photoacoustic imaging, PET, PTT, PDT, gene and drug delivery, and biosensors.	[73,74,89,92]
Nanocages	Galvanic replacement	40–60 nm	Drug delivery, theranostic, targeted therapy, PTT, photoacoustic imaging, two- and three-photon luminescence imaging, SERS.	[27,73,74,89]
Nanostars	Seed-mediated growth	30–60 nm	SERS, CT, optical coherence tomography, fluorescence and photoacoustic imaging, PTT, drug delivery, PDT, and bioimaging.	[73,74,93,94]
Nanoclusters	Chemical reduction and phase transition	1–2 nm	Detection of metal ions, proteins, bacteria, and small molecules, cell imaging, CT imaging, targeted drug delivery, gene delivery, antimicrobial agent, fluorescence imaging, and biosensors.	[95–98]
Nanoflowers	Seed-mediated growth	50–100 nm	SERS, biosensors, PTT, cancer treatment, multimodal imaging, fluorescence imaging, targeted drug delivery.	[99,100]
Nanopyramids	Seed-mediated growth	60–150 nm	PTT, PDT, SERS, optical coherence tomography, bioimaging, and theranostics.	[88,94,101–106]
Nanoshells	Silica template seed-mediated growth	15 nm	Drug delivery, PTT, biomarker detection, antimicrobial agent.	[70,73,74,107]

fluorescence imaging, and photoacoustic imaging. Gold nanostructures also play a crucial role in theranostic platforms, which integrate diagnostics and treatment. Therefore, gold nanostructures are indispensable tools across various scientific and medical disciplines [86,87].

The versatile physicochemical properties of GNPs, influenced by their, size, composition, surface charge, and morphology, make them ideal for radiosensitizers, drug delivery, PTT, and imaging agents [108]. GNP size plays a crucial role in drug delivery efficacy, ensuring they can carry an appropriate amount of drug to target cells and traverse physiological barriers. GNPs with diameters under 100 nm can freely enter cells, while those smaller than 20 nm can rapidly navigate blood vessels. Furthermore, GNPs under 150 nm easily penetrate endothelial barriers, while those exceeding 250 nm are filtered out by the spleen. Size optimization also affects GNP's surface plasmon resonance (SPR) phenomenon, influencing properties such as photothermal ability. Moreover, GNPs with sizes below 3 nm tend to exhibit luminescence in the red to near-infrared region, aiding in diagnosing cancer cells, including metastases [109]. Regarding GNP's surface charge, a net positive or negative charge is preferable to prevent aggregation and facilitate binding with oppositely charged molecules. However, for enhanced *in vivo* biocompatibility, GNPs with reduced surface charges are preferred, often achieved through coatings such as polyethylene glycol (PEG). In this context, bovine serum albumin enhances biocompatibility and facilitates active tumor targeting by leveraging its affinity for specific cell receptors [31,109]. The shape of GNPs profoundly influences cancer therapy and imaging, impacting treatment efficacy and imaging sensitivity. Different shapes exhibit distinct SPR properties, affecting their ability to convert light energy into heat for photothermal therapy. GNP's shape significantly influences their performance as contrast agents in various imaging modalities, with shapes like nanorods or nanocages enhancing contrast in techniques such as SERS or CT. Biological interactions are also shaped by GNP's morphology, affecting cellular uptake, biodistribution, and targeting within tumors. Overall, understanding and optimizing the physicochemical properties of GNPs is crucial for developing more effective nanomedicines and imaging agents [27].

4. Therapeutic applications of gold nanostructures in melanoma

4.1. Photothermal therapy (PTT)

Photothermal therapy (PTT) presents a noninvasive approach to cancer treatment, leveraging materials with exceptional photothermal conversion efficiency that generate hyperthermia upon irradiation, typically by a laser [23,89]. Hyperthermia can provoke apoptosis in human malignant melanoma cells. When applied with clinically relevant drugs for malignant melanoma at controlled temperatures (40–43 °C), it can induce intrinsic or extrinsic endoplasmic reticulum (ER)-mediated apoptosis [110,111]. Unlike other anti-tumor therapies such as immunotherapy, radiotherapy, and molecular-targeted therapies, which often entail systemic adverse effects, PTT offers a more localized approach with fewer side effects [112]. Research has explored the mechanisms underlying PTT-induced cell death mediated by gold nanorods, revealing temperature-dependent necroptosis in addition to apoptosis and necrosis [113]. Moreover, PTT exhibits the potential to trigger a synergistic immune response termed the abscopal effect or SYMPHONY (Synergistic Immuno Photothermal Nanotherapy), leading to the regression of untreated distant tumors following PTT of the primary tumor [114]. PTT can also stimulate antitumor T cell responses by upregulating proinflammatory cytokines and chemokines, although its impact alone is limited. Combining systemic immunotherapy, such as adoptive transfer of specific T cells following PTT by gold nanoshells, effectively inhibits primary and distant tumor growth in B16F10 melanoma [115].

Gold nanostructures have garnered significant attention in PTT due to their unique optical properties, including localized surface

plasmon resonance (LSPR) and high biocompatibility. Gold nanostructures can efficiently absorb NIR light and convert it into heat, a property exploited for PTT. Their ease of functionalization with tumor-targeting recognition elements allows them to accumulate selectively in tumor tissues, leading to precise tumor cell destruction [89]. For instance, green-synthesized spherical GNPs coated with glucose for enhanced stability and internalization exhibited efficient antitumor activity and excellent biocompatibility in murine B16F10 and human SK-MEL-28 melanoma cells [116]. Gold nanostructures reduce the laser power threshold required for photothermal cell ablation, minimizing damage to healthy tissues and amplifying therapeutic efficacy through conjugation with various biomolecules and drugs [117]. For efficient photothermal effects, gold nanostructures should ideally possess specific attributes, including small size (outer diameter of 30–60 nm), as well as strong, narrow, and tunable NIR absorption within the range of $\lambda =$ 650–850 nm (for superficial solid tumors) to $\lambda =$ 950–1350 nm (for deeper penetration) [118]. Coating with PEG acts as a stabilizer and reduces the adsorption of serum proteins, leading to better biocompatibility [26]. For example, utilizing PEGylated GNRs, Light-emitting diode (LED)-based PTT demonstrated enhanced animal survival rates and reduced overall tumor volumes in murine B16F10 melanoma models [119]. It's important to highlight that clinical trials involving GNPs are currently underway for PTT targeting prostate and lung cancer (Table 3). Given that melanoma shares a similarly superficial nature [120], it is reasonable to recognize the significant potential of GNPs for use in melanoma.

PTT using gold nanostructures for melanoma treatment has shown promise, but it also comes with some challenges and limitations. Melanoma is known for its genetic and phenotypic heterogeneity. Different melanoma subtypes and stages may respond differently to PTT. Developing a standardized approach that effectively targets various melanoma types is challenging. In addition, NIR light commonly used for PTT due to its tissue-penetrating capabilities, has limitations in reaching deeply seated tumors. Superficial tumors are more accessible and responsive to PTT, while deep tumors may not receive sufficient light energy for effective treatment. Moreover, ensuring uniform heating of the tumor tissue is challenging. Variations in nanoparticle distribution, tissue optical properties, and heat dissipation can lead to uneven temperature distribution within the tumor, potentially leaving some regions undertreated. Finally, PTT-induced hyperthermia can trigger an inflammatory response that may result in discomfort, pain, or even adverse effects in some patients. Managing these side effects is essential for patient comfort and safety [86]. In summary, PTT using gold nanostructures offers a promising noninvasive approach by exploiting their unique optical properties and preferential accumulation in tumor tissue to treat melanoma through hyperthermia induced by laser irradiation. Nonetheless, challenges persist, such as the heterogeneous nature of melanoma, difficulties in accessing deeply located tumors, and ensuring uniform heating of tumor tissue, indicating the need for further research and optimization for clinical translation. A brief overview of gold nanostructure application in melanoma is summarized in Table 4.

4.2. Photodynamic therapy (PDT)

PDT is a two-step procedure involving the initial administration of a photosensitizing agent (PS), followed by its activation through visible light or NIR irradiation, often using a laser. This process generates reactive oxygen species (ROS), ultimately destroying tumor cells through phototoxic effects. PDT represents a minimally invasive and selective approach to cancer treatment. Mechanistically, the excited PS transfers its energy to surrounding biomolecules or directly to oxygen molecules, producing ROS. The ROS induction can trigger various cellular responses, including apoptosis, autophagy, and necrosis [156,157]. Despite the selectivity of PSs, they can still accumulate in healthy tissues, causing undesirable side effects and reducing treatment efficacy. Immune system clearance further hampers PS uptake *in vivo* [122]. The use of GNPs improves PS uptake due to increased selectivity and the ability of stabilized gold nanostructures to evade the immune system. Conjugating tumor-targeting ligands with GNP-PS complexes, alongside the potential of GNPs for PTT, enhances treatment efficacy by amplifying cellular damage [158]. Additionally, GNPs can enhance the conversion efficiency and ROS production through their SPR phenomenon [123].

Phthalocyanine (*Pc*) derivatives have garnered attention for GNP-mediated melanoma treatment. They possess absorption properties in the red to NIR region, high extinction coefficients in the far-infrared (~670 nm), ease of functionalization, and the ability to engage in multiple photochemical and photophysical pathways for ROS generation [158]. For instance, PEG-ZnPc-GNPs and ZnPc disulfide-GNPs have demonstrated effective photodynamic destruction of amelanotic melanoma tumors, with the highest efficacy observed when irradiation was performed 3 h post-PS injection. Remarkably, PEG-ZnPc-GNPs achieved a 40 % cure rate in mice without tumor regrowth [121]. The incorporation of zinc into the *Pc* macrocyclic structure induces both apoptotic and necrotic pathways in melanoma cells following light irradiation [159]. In A375 melanoma cell lines, zinc phthalocyanine tetra-sulphonic acid (ZnPcS4), a safer and more water-soluble derivative, was conjugated onto amine-functionalized GNPs and linked with anti-Melanoma Inhibitory Activity (anti-MIA) antibodies (specifically targeting melanoma cells). This approach significantly enhanced the effectiveness of PDT in melanoma treatment [122]. Similarly, aluminum phthalocyanine chloride (AlPcS4Cl) conjugation on GNPs in A375 melanoma cells exhibited increased cytotoxicity and reduced melanoma stem cell proliferation compared to unconjugated AlPcS4Cl [123].

A significant limitation of PDT in melanoma arises from melanin pigmentation, creating a formidable barrier that hinders the penetration of laser light into tumor cells [123]. This inherent limitation restricts the use of PDT primarily to amelanotic melanoma cases or necessitates its combination with other melanoma treatments, such as targeted drug delivery and PTT, to effectively manage pigmented melanoma [121,160]. To overcome this challenge, researchers often resort to PDT in conjunction with other therapies, like targeted drug delivery and PTT, to enhance its efficacy in pigmented melanoma scenarios [122,158,159]. Selecting an appropriate PS is crucial, as it determines the laser absorption peak. The use of longer wavelengths becomes imperative to ensure deeper light penetration through melanin-rich tumor cells [122]. A creative study tried to use sunlight as the PDT light source via a heterogeneous nanocomposite. To optimize the wavelength to be shorter than 729 nm, gold nanoclusters, due to their narrow band gap energy, were

Table 3
Clinical trials involving gold nanoparticles (GNPs). Data collected from the ClinicalTrials.gov database (Accessed on Mar 10, 2024).

Trial Number	Year	Trial Title	Phase	Condition	Intervention	Status
NCT05816512	2023	Antimicrobial Efficacy of Biogenic Gold Nano Particle from Pelargonium Graveolens Leaves Extract Mouthwash for Children	Not Applicable	Dental Caries, Gingivitis, Periodontitis	Gold Nanoparticle from Pelargonium Graveolens Mouthwash	Recruiting
NCT02755870	2015–2016	Therapeutic Nanocatalysis to Slow Disease Progression of Amyotrophic Lateral Sclerosis	Phase 1	Healthy Volunteers - Male and Female	CNM-Au8 (a catalytically active gold nanocrystal neuroprotective agent), Placebo	Completed
NCT04098406	2019–2021	Therapeutic Nanocatalysis to Slow Disease Progression of Amyotrophic Lateral Sclerosis (ALS)	Phase 2	Amyotrophic Lateral Sclerosis	CNM-Au8	Completed
NCT05299658	2021	An Open-Label Extension for the Phase 2 Study in Early Symptomatic Amyotrophic Lateral Sclerosis	Phase 2	Amyotrophic Lateral Sclerosis	CNM-Au8	Active, not recruiting
NCT03993171	2019	31P-MRS Imaging to Assess the Effects of CNM-Au8 on Impaired Neuronal Redox State in Multiple Sclerosis.	Phase 2	Multiple Sclerosis	CNM-Au8	Recruiting
NCT03815916	2019–2021	31P-MRS Imaging to Assess the Effects of CNM-Au8 on Impaired Neuronal Redox State in Parkinson's Disease	Phase 2	Parkinson's Disease	CNM-Au8	Completed
NCT04414345	2022–2023	HEALEY ALS Platform Trial - Regimen C CNM-Au8	Phase 2	Amyotrophic Lateral Sclerosis	CNM-Au8, Placebo	Completed
NCT04081714	2019	Intermediate Expanded Access Protocol for ALS	Not applicable	Amyotrophic Lateral Sclerosis	CNM-Au8	Ongoing
NCT04297683	2020	HEALEY ALS Platform Trial - Master Protocol	Phase2/3	Amyotrophic Lateral Sclerosis	Zilucoplan, Verdiperstat, CNM-Au8, Pridopidine, SLS-005 Trehalose, ABBV-CLS-7262, DNL343	Recruiting
NCT03536559	2018–2022	Nanocrystalline Gold to Treat Remyelination Failure in Chronic Optic Neuropathy in Multiple Sclerosis	Phase 2	Multiple Sclerosis	CNM-Au8	Completed
NCT04626921	2020–2023	A Multi-Center, Open-Label Long-Term Extension Study of CNM-Au8 in Patients with Stable Relapsing Multiple Sclerosis	Phase2/3	Multiple Sclerosis	CNM-Au8	Completed
NCT04907422	2018–2021	Diagnostic and Prognostic Accuracy of Gold Nanoparticles in Salivary Gland Tumors	Not applicable	Carcinoma Ex Pleomorphic Adenoma of Salivary Glands, Pleomorphic Adenoma of Salivary Glands	Diagnostic Test: CD24-Gold Nanocomposite expression using Real-time quantitative polymerase chain reaction, Diagnostic Test: non-conjugated CD24 expression using Real-time quantitative polymerase chain reaction	Completed
NCT01679470	2012–2014	Efficacy Study of AuroLase Therapy in Subjects with Primary and/or Metastatic Lung Tumors	Not applicable	AuroLase Device (NIR source + Silica-gold nanoshells coated with PEG)	Primary and/or Metastatic Lung Tumors	Completed
NCT00848042	2008–2014	Pilot Study of AuroLase Therapy in Refractory and/or Recurrent Tumors of the Head and Neck	Not applicable	AuroLase Therapy	Refractory and/or Recurrent Tumors of the Head and Neck	Completed
NCT02680535	2016–2020	MRI/US Fusion Imaging and Biopsy in Combination with Nanoparticle Directed Focal Therapy for Ablation of Prostate Tissue	Not applicable	AuroLase Therapy	Prostate cancer	Completed
NCT01270139	2007–2016	Plasmonic Nano photothermal Therapy of Atherosclerosis (NANOM-FIM)	Not applicable	Stable Angina, Heart Failure, Atherosclerosis, Multivessel Coronary Artery Disease	Transplantation of iron and Silica-GNPs	Completed
NCT05113862	2022	A Phase-I Study of a Nanoparticle-based Peptide Vaccine Against SARS-CoV-2	Phase 1	SARS-CoV-2	LD Vehicle-GNP, LD PepGNP-Covid19, HD Vehicle-GNP,	Completed

(*continued on next page*)

Table 3 (*continued*)

Trial Number	Year	Trial Title	Phase	Condition	Intervention	Status
NCT00356980	2006–2009	TNF-Bound Colloidal Gold in Treating Patients with Advanced Solid Tumors	Phase 1	Unspecified adult solid tumor	HD PepGNP-Covid19 colloidal gold-bound tumor necrosis factor, pharmacological study	Completed
NCT03020017	2017–2020	NU-0129 in Treating Patients with Recurrent Glioblastoma or Gliosarcoma Undergoing Surgery	Phase 1	Gliosarcoma, Recurrent Glioblastoma	Laboratory Biomarker Analysis, Pharmacological Study, Targeted Molecular Therapy (GNP as a carrier)	Completed
NCT02837094	2016–2019	Enhanced Epidermal Antigen-Specific Immunotherapy Trial −1	Phase 1	Type I diabetes	C19-A3 GNP (ultrasmall-GNPs (less than 5 nm) loaded with a proinsulin-derived peptide)	Completed
NCT05347602	2020	Gold Factor on Knee Joint Health and Function	Not applicable	Arthritis	Gold nanoparticles (GNPs)	Completed

conjugated to titanium dioxide (TiO_2), the PDT agent and graphene were added as a semiconductor with photocatalytic activity. The results showed severe cell death in B16F10 cells and significant tumor growth inhibition in B16F1-tumor-xenograft-bearing mice [124].

Sonodynamic Therapy (SDT) emerges as a promising alternative to PDT, addressing limitations like poor light penetration and skin sensitivity. SDT utilizes low-intensity ultrasound waves and sound-sensitive compounds to induce apoptosis through various mechanisms, including ROS generation and alteration of cellular processes. Nanotechnological advancements, like Titania/gold nanoparticles (TGNPs), enhance SDT efficacy by combining titania's inertness with gold's ROS-enhancing properties. Recent studies show TGNPs' cytotoxicity against C540 melanoma cells, highlighting SDT as a promising approach for cancer therapy [125].

In summary, PDT presents a promising approach to cancer treatment by utilizing light irradiation to activate PS, leading to ROS generation and subsequent tumor cell destruction. While PDT shows potential, challenges like PS distribution in healthy tissues and limited efficacy in pigmented melanoma persist. Moreover, factors such as the depth of light penetration, tumor heterogeneity, and the potential for therapy resistance can impede PDT effectiveness. Integration of GNPs enhances PS uptake and treatment efficacy, particularly in melanoma, where innovative strategies such as combination therapies and nanocomposites are being explored to overcome barriers and improve outcomes. Additionally, SDT emerges as an alternative to address PDT limitations, utilizing low-intensity ultrasound waves and sonosensitizers like GNPs to trigger ROS generation and enhance treatment efficacy, particularly in deep-seated tumors.

4.3. Targeted drug and gene delivery

Due to their unique size, nanoparticles can exploit the distinct characteristics of cancer pathology and molecular biology, resulting in increased uptake and preferential targeting of disease sites compared to conventional therapies [161]. Recent advancements in nanotechnology offer a promising solution for improving drug delivery by addressing issues such as targeted delivery and minimizing collateral damage to healthy tissues [162]. In general, targeted drug or gene delivery can be categorized into two main mechanisms: passive and active drug delivery. Passive therapeutic targeting takes advantage of unique changes in the vasculature of cancerous tissues. Rapid tumor growth often leads to poorly formed and leaky blood vessels. Nanoparticles, due to their size, can pass through these compromised junctions, leading to their gradual accumulation at the tumor site. This phenomenon is known as the enhanced permeation and retention (EPR) effect [163,164] (Fig. 4). Active drug delivery, on the other hand, relies on specific interactions between ligands bound to the carrier and receptors on the target cell. These ligands can be antibodies, proteins, peptides, nucleic acids, sugars, or small molecules like vitamins, while the receptors are typically more abundant in cancer tissues or exclusively present in cancerous cells compared to healthy tissues [163,165]. In this process, nanoparticles are recognized, bind to target cells through ligand-receptor interactions, and are subsequently internalized, minimizing off-target drug release compared to passive targeting approaches. Active targeting can enhance nanoparticle retention and therapeutic payload in cancer tumors by specifically targeting cell membrane receptors that are overexpressed in cancer cell lines [166]. In active targeting, GNPs are linked with an array of active ligands that interact with specific cell surface receptors, effectively ferrying payloads to the desired site and facilitating controlled medication release [167].

GNPs are extensively studied for drug delivery applications in melanoma treatment. For instance, by conjugating the chemotherapeutic agent doxorubicin (DOX) to GNPs, a significant reduction in the IC_{50} of melanoma B16 cells has been achieved. This is attributed to the fact that DOX alone can only enter tumor cells through simple diffusion, whereas DOX-GNPs can also be taken up via endocytosis [126]. Similarly, imatinib mesylate, a kinase inhibitor chemotherapeutic, was encapsulated in layer-by-layer charged polymer-coated GNPs, resulting in a marked reduction in cell growth in the B16F10 melanoma cell line. The polyelectrolyte-coated GNPs, with their higher positive charge density, allowed for anodal iontophoresis and enhanced skin permeation, enabling more effective topical delivery [127]. Sorafenib, another kinase inhibitor, was encapsulated in spherical GNPs, exhibiting potent inhibitory activity against tumor angiogenesis and proliferation, surpassing the effects of sorafenib alone in B16F10 and A375 melanoma cell lines [128].

A recent study proposes a novel strategy for treating melanoma tumors by combining radiotherapy, PTT, and chemotherapy. The

Table 4
GNPs' applications in melanoma.

Application	Carrier	Cargo	Aim	Results	Ref.
Photothermal therapy (PTT)	GNP	gp100-specific T cells	Combination of PTT and immunotherapy in B16F10 cells	Inhibition of primary and distant tumor growth	[115]
	Glu-GNP	–	PTT in B16F10 and SK-MEL-28 cells	Efficient antitumor activity and excellent biocompatibility	[116]
	PEG-GNP	–	LED-based PTT in B16F10 model	Enhanced animal survival rate and reduced overall tumor volume	[119]
Photodynamic therapy (PDT)	PEG-GNPs and GNPs	ZnPc and ZnPc disulfide (photosensitizer)	PDT of B78H1 tumors	Photodynamic destruction	[121]
	Anti-Mia-GNP	ZnPcS4 (photosensitizer)	Targeted PDT in A375 cells	Enhanced effectiveness of PDT	[122]
	GNP	AlPcS4Cl (photosensitizer)	PDT in A375 cells	Increased cytotoxicity and reduced melanoma stem cell proliferation	[123]
	Gold nanocluster-graphene	TiO_2 (photosensitizer)	PDT in B16F10 cells and B16F1 mouse	severe cell death and significant tumor growth inhibition	[124]
Sonodynamic therapy (SDT)	–	TGNPs (Sonosensitizer)	SDT in C540 melanoma cells	Efficient cytotoxicity with higher levels of ROS	[125]
Targeted delivery	GNP	Doxorubicin	Targeted drug delivery to B16 cells	A significant reduction in the IC_{50}	[126]
	Layer-by-layer charged polyelectrolyte-coated GNPs	Imatinib	Targeted drug delivery to B16F10 cells in combination with iontophoresis	Reduction in cell growth, enhanced skin permeation	[127]
	GNP	Sorafenib	Targeted drug delivery to B16F10 and A375 cells	Inhibition of tumor angiogenesis and proliferation	[128]
	GNR-188Re	Paclitaxel	Targeted drug delivery combined with PTT and radiotherapy in B16–F10 melanoma-bearing C57BL/6 mice	Effective Multimodal Therapy, Inhibition of Tumor Growth without significant side effects	[129]
	Anti-Her2-GNP	–	Targeted drug delivery to G631 cells	Apoptosis of melanoma cells with high affinity and selectivity	[130]
	[Nle4, D-Phe7] α-MSH -PEG-GNP	–	Targeted PTT in B16F10 cells	Receptor-mediated active targeting and photothermal ablation	[91]
	Anti-Her2-GNP	–	Targeted delivery combined with non-thermal atmospheric pressure plasma in G361 cells	Cell destruction via degradation of Focal Adhesion Kinase (FAK)	[131]
	Anti-pFAK-GNP	–	Targeted delivery combined with non-thermal atmospheric pressure plasma in G361 cells	Selective and effective destruction of melanoma cells	[132]
	GNP-Apt^{IgG}	anti-BRAFV600E	Targeted delivery to melanoma A2058, Malme-3 M, and SK-MEL-2 cells	Selective inhibition of mutant melanoma cells, reduced proliferation, increased apoptosis, and no observed toxicity	[133]
	Folic acid conjugated with cysteamine-GNP	–	Targeted delivery to melanoma combined with ultrasound	Enhanced death of melanoma cells	[134]
	GNP clusters	Doxorubicin	Targeted delivery combined with PTT to B16 melanoma	Efficient drug loading, tumor cell internalization, and light-triggered drug release, effective tumor suppression without adverse effects	[135]
	E. coli OMV-coated β-CD–ADA-GNP		Targeted photothermal and immunotherapy in B16 mouse	Inhibition of tumor growth, immunogenic cell death induction, synergy with anti-PD-1	[136]
	GNP	miR-21-3p anti-PD1	Targeted gene and immunotherapy in human cell lines such as A375 and mouse B16F10	Promotion of IFN-γ-driven ferroptosis and synergy with anti-PD-1	[137]
	TAT conjugated cationic GNPs	pDNAs encoding miRNA-221 inhibitor gene (Mi221)	targeted gene delivery to B16F10	Efficient skin penetration and effective transfection	[138]

(*continued on next page*)

Table 4 (*continued*)

Application	Carrier	Cargo	Aim	Results	Ref.
	layer-by-layer chitosan-coated GNP	STAT3 siRNA	Targeted gene delivery to B16F10	Decreases cancer cell viability via the induction of apoptosis	[139]
	layer-by-layer chitosan-coated GNP	STAT3 siRNA And imatinib	Targeted gene and drug delivery to B16F10	Substantial reduction in tumor weight and volume, suppression of STAT3 protein expression	[140]
Nanovaccine	GNPs linked to a shikimoyl ligand	Plasmid DNA encoding the melanoma antigen (MART1)	DNA vaccine in C57BL/6J mice and B16F10 cell line	Long-lasting anti-melanoma immune response, prophylactic and inhibitory therapeutic effects	[141, 142]
	Non-covalent glycosylated GNP within a β-CD self-assembly	OVA-derived peptide and a B16F10 neoantigen	Peptide-based antigen vaccine in C57BL6/J model	Effective generation of antibodies and enhanced therapeutic response against melanoma	[143]
	GNPs within an α-CD hydrogel system	PEGylated OVA-derived peptide in conjunction with CpG adjuvant	Peptide-based antigen vaccine in C57BL/6 mice	Significant inhibition of tumor progression and increased survival rates with long half-life and high efficacy	[144]
	Liposome-coated gold nanocages	aCD11c antibody, adjuvant MPLA, melanoma antigen peptide TRP2	Peptide-based antigen vaccine in B16–F10 prophylactic and lung metastasis models	remarkable antitumor immune response, and effective restrain of tumor growth and metastasis	[85]
Imaging	GNP	–	CT label for melanoma-specific T cell receptor	Enhanced image of substantial T cell accumulation at the tumor sites	[145]
	RGD-gold nanoclusters	–	Fluorescence imaging of A375 melanoma cells	Efficient fluorescent activity with good biocompatibility	[98]
	NDP-MSH-GNP	–	Contrast agent for Photoacoustic (PA) imaging	Contrast enhancement	[146]
	PFH-GNPs conjugated with MAGE-1 mAb	–	dual-mode contrast agent for PA/ultrasound imaging	Increased accumulation and enhanced resolution	[147]
	Positively charged GNPs	–	planar solid SERS substrate	Differentiation of melanocytes and melanoma cells	[148]
	GNPs	4-mercaptobenzoic acid, 2,7-mercapto-4-methyl coumarin, and 4-mercapto-3-nitrobenzoic acid (as Raman reporters)	PCR-based SERS	Accurate identification of melanoma mutation carriers with high specificity	[149]
	PEG-GNPs conjugated with NDP-MSH and ^{64}Cu	–	PET in B16F10 cells	Sensitive and precise detection of specific melanocortin receptor MC1R	[150]
	^{89}Zr-GNPs	–	PET in B16F10 cells	Tumor contrast	[151]
Theranostics	PEG-GNP-TAT	Cy5-tagged caspase-3 specific peptides (Cy5-DEVD) (apoptotic and optical agent)	Theranostic system (B16F10 cells)	Apoptosis of melanoma cells, PTT, fluorescence	[152]
	anti-GD2-GNPs combined with single-wall carbon nanotubes (SWCNT)	–	hybrid theranostic system (UACC903 cells)	Nearly 100 % of melanoma cells rendered nonviable after irradiation with NIR monitored by selective two-photon imaging	[153]
	GNPs functionalized with folic acid and transferrin	Dabrafenib	Theranostic system	Efficient and controlled release of dabrafenib in melanoma, confirmed by SERS	[154]
	liposomal layer-coated GNPs modified with DC-specific antibody aCD11c	MPLA (adjuvant), melanoma antigen peptide TRP2	Theranostic nanovaccine targeting B16F10 cells	Efficient inhibition of tumor growth by enhancing the immune response, monitored by fluorescence and photoacoustic imaging.	[85]
	liposomal layer-coated GNPs decorated with anti-MUC18 single-chain antibody	4-mercaptobenzoic acid (as a Raman tag)	Theranostic system (A375 cells)	Selective melanoma diagnosis using SERS with effective remote-controlled PTT	[78]
	PFH-GNPs conjugated with MAGE-1 mAb		Theranostic system (B16F10 cells)	Effective PTT and optical droplet vaporization (ODV) therapy as well as reduced toxicity, monitored using ultrasound imaging	[143]
	GNPs coated with anti-DR5 decorated BSA-gold nanocluster	Dacarbazine, ISQ (photosensitizer and Raman reporter)	Theranostic system (A375 cells)	Synergistic cell apoptosis via chemotherapy, PTT, PDT, monitored and confirmed by SERS	[155]

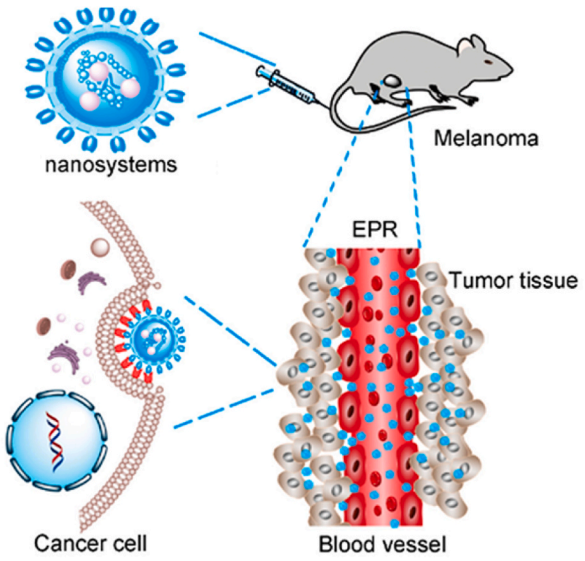

Fig. 4. Nanocarrier-based drug delivery for melanoma therapeutics. Adapted from Song et al., Int. J. Mol. Sci. 2021, 22(4), 1873; DOI: https://doi.org/10.3390/ijms22041873. Licensed under a Creative Commons Attribution 4.0 International License (CC BY). Stepwise illustration of enhanced permeability and retention (EPR) effect of nanoparticles for cancer therapy [168].

study investigates the synergistic effects of these therapies using GNRs optimized to absorb NIR light efficiently. These GNRs are radiolabeled with the therapeutic isotope rhenium-188 (188Re) to enhance their therapeutic potential. Additionally, paclitaxel (PTX) is incorporated to maximize treatment efficiency. The *in vivo* introduction of the developed system demonstrates effective multimodal therapy, resulting in synergistic chemo-photothermal effects from different therapies. Evaluation of combined tumor therapy in B16–F10 melanoma-bearing C57BL/6 mice shows promising results in inhibiting tumor growth without significant side effects on healthy tissues [129].

Monoclonal antibodies (mAbs) play a pivotal role in active targeted drug delivery due to the overexpression of various antigens on the surface of tumor cells. Conjugating mAbs with nanoparticles mitigates their primary drawbacks, such as rapid elimination, while enhancing their targeting capabilities [169]. For instance, anti-human epidermal growth factor receptor 2 (anti-HER2) is a notable mAb that, when linked with GNPs, exhibits a strong affinity for and selectively binds to melanoma G361 cells, triggering apoptosis in these cancerous cells [130]. α-Melanocyte Stimulating Hormone (MSH) is an anti-inflammatory cytokine with aberrant activity in melanoma, primarily contributing to the overexpression of melanocortin type-1 receptor (MC1R) on melanoma cells that presents a promising avenue for targeted melanoma therapy [170,171]. A more potent analog of MSH, [Nle4, D-Phe7] α-MSH or NDP-MSH, was attached to PEGylated hollow gold nanospheres for receptor-mediated active targeting PTT in the B16F10 melanoma cell line. Histologic and [^{18}F] fluorodeoxyglucose PET evaluation following NIR irradiation confirmed the successful photothermal ablation [91]. Furthermore, GNPs conjugated with anti-HER2 (also known as anti-NEU) have been combined with non-thermal atmospheric pressure plasma to target G361 melanoma cells. The results demonstrated that cell destruction via NEU phosphorylation led to the degradation of Focal Adhesion Kinase (FAK), a kinase crucial in the proliferation, migration, adhesion, and differentiation of melanoma cells [131]. In another study, the conjugation of phosphorylated-FAK (*p*-FAK) antibodies with GNPs (*p*-FAK-GNP) has demonstrated remarkable selectivity in targeting G361 melanoma cells. Non-thermal atmospheric pressure plasma was employed to activate the GNPs. The results revealed highly selective and effective destruction of cancer cells, with a mortality rate more than three times higher than treatment with plasma alone [132]. In addition to mAbs, small molecules such as folic acid can target cancer cells, specifically melanoma. This is because cancer cells, including melanoma, have an increased demand for folic acid due to their rapid growth and proliferation. To assess the capacity of GNPs to boost acoustic cavitation, researchers attached cysteamine and folic acid to these particles. Subsequently, they exposed this composition to ultrasound, and the findings indicated a decrease in the viability of melanoma cells. This effect depended on the concentration and size of the particles [134].

Aptamers (Apt), obtained through the systematic evolution of ligands by exponential enrichment (SELEX) technology, are short DNA or RNA molecules known for their high specificity and affinity to various targets, including metal ions, small molecules, proteins, cells, and tissues. They offer advantages like thermal stability, smaller molecular weight, ease of modification, and minimal batch-to-batch variation compared to conventional antibodies [172]. A specific DNA aptamer against the Fc region of immunoglobulin G (AptIgG)-conjugated GNPs have been utilized for intracellular antibody delivery. This platform enables the loading of various antibodies onto GNP-AptIgG through simple mixing. Effective cytosolic delivery of antibodies to clinically significant mutant proteins via scavenger receptors and caveolae-mediated endocytosis has been demonstrated, particularly in cancer cells expressing BRAFV600E. In vivo, experiments involving subcutaneous injection of GNP-AptIgG-aBRAFV600E into xenografted melanoma tumors expressing BRAFV600E regressed tumor in mice by inhibiting proliferation and inducing apoptosis. Further investigation in xenografted mice revealed selective inhibition of melanoma cells bearing the BRAFV600E mutation after treatment with GNP-AptIgG-aBRAFV600E.

Immunohistochemical analysis of tumor sections demonstrated reduced proliferation and increased apoptosis, as evidenced by decreased levels of the pro-survival protein BCL2 and increased levels of the pro-apoptotic protein Bcl-2 homologous antagonist/killer (BAK). Importantly, GNP-AptIgG-aBRAFV600E treatment did not induce toxicity, as indicated by unchanged body and organ weights and minimal pathological changes. These findings highlight the successful delivery of anti-BRAFV600E antibody by the GNP-AptIgG carrier into tumors, resulting in potent suppression of tumor growth through apoptosis induction and proliferation inhibition in melanoma cancer cells [133].

Cell-based drug delivery is a method that uses endogenous cells as carriers to improve therapeutic efficiency by evading the clearance system. Immune cell-mediated delivery could show great tumor-targeting efficiency. However, the immunosuppressive tumor microenvironment limits immune cell recruitment. To overcome this obstacle, a recent study used β-cyclodextrin (β-CD)–modified GNPs conjugated with adamantane (ADA) and coated with outer membrane vesicles (OMVs) of Escherichia coli (E. coli) in B16 mouse melanoma model. The E. coli OMVs attract phagocytes, leading to the intracellular degradation of the nanocomposite, and the subsequent self-assembly of GNPs via β-CD–ADA host-guest interactions inside the immune cells. This self-assembly prevents the leakage of the GNPs from the carrier cells while they deliver them to the tumor tissues. Initially, PTT was performed to induce tissue damage and trigger inflammation. This inflammation acts as positive feedback, resulting in increased recruitment of immune cells carrying the GNPs, leading to enhanced accumulation at the tumor site. Subsequently, a secondary round of PTT is conducted. Results showed negligible systemic toxicity with the secondary PTT to be highly effective, as well as improved immunotherapy and a synergistic response when administered with the immune checkpoint blocker, atezolizumab [136].

Hyaluronic acid (HA) is implicated in melanoma progression, with elevated CD44 levels associated with advanced cancer stages and poor prognosis. Melanoma cells produce HA during early tumorigenesis, while activated stromal fibroblasts predominantly produce HA in later stages, exposing melanoma cells to self-produced and stroma-derived HA throughout tumor development. HA in melanoma promotes cell proliferation, adhesion, motility, invasion, and metastasis [173]. A recent study introduced adaptable gold nanoparticle clusters (GNCs) for synergistic photothermal chemotherapy in B16 melanoma cells. This approach involved linking 2-naphthaleneacetic acid (2-NAA) to HA to create an amphiphilic multivalent guest molecule (HAN), followed by attaching β-cyclodextrin (β-CD) to small GNPs to accommodate the guest molecule and therapeutic drug. The resulting DOX@GNCs exhibited NIR light sensitivity, selectively releasing the chemotherapy drug DOX, inducing cancer cell apoptosis through combined photothermal and chemotherapy effects. In vitro and *in vivo* studies demonstrated promising therapeutic outcomes, with DOX@GNCs effectively suppressing tumor growth under clinically approved laser power without recurrence, inflammation, or organ damage, suggesting the potential of GNC-based nanoplatforms for versatile PTT-chemo synergistic therapy [135].

MicroRNAs (miRNAs) are endogenous small non-coding RNAs that negatively regulate gene expression via interacting with the target mRNA, leading to ferroptosis. miRNAs-based mimic drugs have been revealed to have high antitumor potency. MiRNAs play a crucial role in various cellular events associated with melanoma cancer, such as genesis, cell cycle regulation, tumor growth, proliferation, migration, invasion, drug resistance, and apoptotic induction. Downregulated miRNAs, including miR-211, miR-196a, miR-

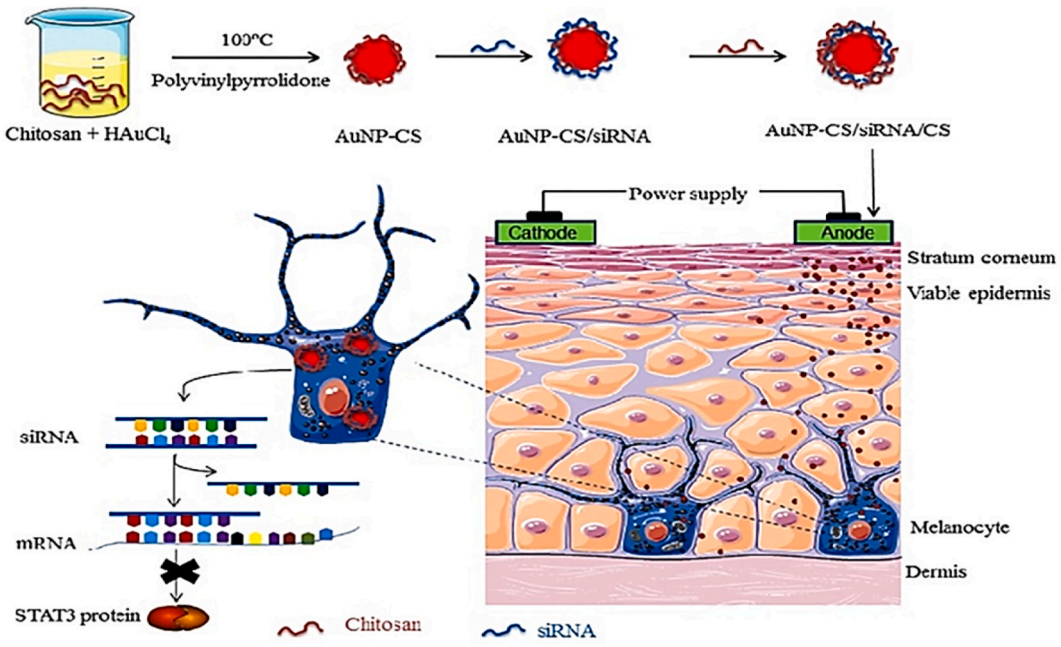

Fig. 5. Schematic illustration of layer-by-layer assembly GNPs (LbL-GNPs) composed of chitosan (CS) coated GNPs (AuNP-CS) and STAT3 siRNA with additional CS coating for iontophoretic transdermal delivery and STAT3 gene knockdown in B16F10 murine melanoma cells. LbL-GNPs are stable in ionic media. CS reduces overall toxicity. AuNP-CS entraps STAT3 siRNA and improves stability and cell uptake. Iontophoresis promotes localized skin penetration [139].

21, miR-124, miR-29c, and miR-210, can influence the biological processes of melanoma cancer cells [174]. In a recent study, miR-21–3p, a mature form of the melanoma oncogenic factor miR-21, was delivered using GNPs combined with anti-programmed cell death protein-1(anti-PD-1) antibody in both human and mouse melanoma cell lines. The results showed that miR-21–3p promotes IFN-γ-driven ferroptosis and sensitizes melanoma cells to anti-PD-1 immunotherapy, and a robust increase in the level of miR-21–3 p in tumors was observed due to the systemic delivery of miR-21–3 p using GNPs alongside with low toxicity [137].

Small interfering RNA (siRNA) has emerged as a potent strategy for targeted cancer therapy, facilitated by employing stabilized nanocarriers. siRNA functions by effectively silencing post-transcriptional genes, either through the inhibition of translation or the cleavage of mRNA [175]. In the context of melanoma therapy, Signal Transducer and Activator of Transcription 3 (STAT3) is an excessively expressed protein that inhibits apoptosis and promotes proliferation. Researchers have harnessed the potential of Layer-by-layer assembly GNPs (LbL-GNPs) as carriers for iontophoretic delivery of STAT3 siRNA to B16F10 murine melanoma cells (Fig. 5). The results demonstrate a notable decrease in cancer cell viability attributable to the induction of apoptosis [139]. Moreover, this method exhibits promise when combined with chemotherapeutics like imatinib, as LbL-GNPs can co-deliver STAT3 siRNA and imatinib. This synergistic approach has led to a substantial reduction in tumor weight, and volume, and a significant suppression of STAT3 protein expression in B16F10 cells, surpassing the individual effects of imatinib or siRNA alone [140]. In conclusion, LbL-GNPs for targeted siRNA delivery, particularly in conjunction with chemotherapy, holds considerable potential as a promising avenue in melanoma therapy.

When it comes to the use of DNA, plasmid DNA (pDNA) is a preferred option for gene therapy due to its superior stability compared to other forms [138]. Transdermal delivery in melanoma is considered an efficient method that bypasses the potential toxicities associated with systemic delivery. However, when it comes to delivering macromolecules, chemical enhancers are not sufficiently helpful, and the use of physical enhancers often leads to a disruption of skin barrier properties [176]. In a study, cationic gold nanoparticles (GNPs) conjugated with a cell-penetrating peptide named Trans-Activator of Transcription (TAT) were used to topically deliver plasmid DNAs encoding the miRNA-221 inhibitor gene (Mi221) to B16F10 melanoma cells. The results demonstrated that the nanocomplex could penetrate the stratum corneum without requiring additional enhancements. Furthermore, effective transfection was achieved through highly efficient uptake and targeting of plasmid DNA into melanoma cells [138].

To summarize, GNPs exploit unique cancer characteristics to enhance the uptake and targeting of various chemotherapeutic agents, monoclonal antibodies (mAbs), and nucleic acid-based therapies such as microRNAs (miRNAs) and small interfering RNA (siRNA), resulting in potent anti-tumor effects. The versatility and adaptability of GNPs in targeted delivery underscore their potential as powerful tools in the fight against melanoma.

4.4. Nanovaccines

Nanovaccines typically refer to vaccines utilizing nanoparticles either as adjuvants or carriers. Due to their nano-scale sizes, they provide superior stability to antigens and can stimulate the immune system effectively. This encapsulation of antigens or adjuvants also leads to a notable reduction in systemic toxicity. In the context of cancer, nanovaccines represent immunotherapy-driven agents primarily focused on triggering anti-tumor immune responses while reducing immunosuppressive reactions within the tumor microenvironment [177–179]. The immune response initiates when antigen-presenting cells (APCs), such as dendritic cells (DCs), capture and process a pathogenic antigen. Subsequently, the APC carrying the antigen migrates to nearby lymph nodes, where antigen fragments are delivered to T cells, activating T cells and immune responses. Sipuleucel-T (Provenge) for prostate cancer, approved by the FDA in 2010, stands as the sole nanovaccine that has received regulatory approval. Proposed factors contributing to the failure of nanovaccines to reach the market include the absence of suitable adjuvants and antigens, ineffective antigen presentation, and the immunosuppressive effects of tumors [180].

4.4.1. DNA vaccines

DNA vaccines based on DCs incorporate a plasmid DNA encoding immunogenic tumor-associated antigens. Cationic GNPs have become one of the promising carriers for delivering DNA vaccines *in vivo* because, in addition to their usual benefits such as easy synthesis and functionalization, they enhance DC transfection so that there would be no need to go through the expensive, time-consuming process of isolating autologous DCs [141,142]. In a study involving C57BL/6J mice and a B16F10 melanoma cell line, a combination of gold nanoparticles (GNPs) linked to a shikimoyl ligand via a 6-amino hexane thiol spacer and a DNA vaccine encoding the Melanoma Antigen Recognized by T Cells 1 (MART1) exhibited a long-lasting anti-melanoma immune response. It was demonstrated that mannose-mimicking groups featuring shikimoyl head groups exhibited superior effectiveness in transfecting DCs [141], and had significant prophylactic and inhibitory therapeutic effects [142].

4.4.2. Peptide-based antigen vaccines

Peptide-based antigen vaccines have gained popularity recently due to their low toxicity and convenient storage characteristics. To enhance the immunogenicity and lower the rapid systemic elimination, researchers have explored nanocarriers [143]. GNPs have emerged as valuable tools in this context, as they can stabilize peptides, promote the accumulation of vaccines in lymph nodes, and facilitate uptake by APCs. This approach may entail host-guest complexation to encapsulate the peptide, utilizing versatile and biocompatible materials such as cyclodextrins (CD) [143,144]. In one study, a non-covalent glycosylated GNP-peptide nanovaccine, based on a β-CD host-guest self-assembly, was developed and tested on a C57BL6/J melanoma model using an Ovalbumin (OVA)--derived peptide and a B16F10 neoantigen. This nanovaccine effectively generated antibodies, enhanced the therapeutic response against melanoma, and demonstrated the potential of such carriers as platforms for transporting T cell-independent antigens [143]. In

another study, an OVA-derived peptide was PEGylated and bound to GNPs within an α-CD hydrogel system, in conjunction with cytosine–guanosine dinucleotide (CpG) adjuvant. This hydrogel exhibited a long half-life and high efficacy, significantly inhibiting tumor progression and increased survival rates in C57BL/6 mice with B16-OVA melanoma (Fig. 6) [144]. Liang et al. developed a flexible nanovaccine using liposome-coated gold nanocages (GNCs) decorated with aCD11c, a dendritic cell-specific antibody. This strategy facilitated the targeted delivery of the adjuvant MPLA and the melanoma antigen peptide Tyrosinase-related protein 2 (TRP2) to stimulate dendritic cell activation and maturation. As a result, this approach enhanced specific T lymphocyte responses against tumors. *In vivo* experimental results demonstrated that these precisely targeted GNCs exhibited a notable antitumor immune response, effectively suppressing tumor growth and metastasis in B16–F10 prophylactic and lung metastasis models [85]. In conclusion, GNPs have shown promise in enhancing peptide-based antigen vaccines, offering significant potential for improving vaccine stability, efficacy, and therapeutic outcomes in melanoma (see Fig. 7).

4.4.3. Other vaccines

We propose further research on TLRs as adjuvants in GNP-based nanovaccines for melanoma. Toll-like receptors (TLRs) play a crucial role in innate immunity, recognizing pathogen-associated molecular patterns (PAMPs) and activating signaling pathways that induce inflammatory responses, contributing to the body's defense against pathogens [182]. Incorporating TLR ligands as adjuvants into vaccines not only boosts antigen-specific immune responses but also strengthens the link between innate and adaptive immunity, thereby augmenting antigen presentation to T cells by antigen-presenting cells, ultimately contributing to the design of novel vaccine strategies by antigen-specific T-cell development and the maintenance of memory [182]. Monophosphoryl lipid A (MPLA), a derivative of Lipopolysaccharides (LPS) activating TLR4, marked the first TLR used as an adjuvant. AS04, a vaccine adjuvant comprising aluminum and MPL, was licensed and integrated into the Cervarix vaccine [182]. Polyinosinic:polycytidylic acid (Poly I:C), a TLR-3 agonist, displays anti-cancer activity and induces pro-inflammatory cytokines, serving as a dendritic cell maturation agent to stimulate Type 1 T helper (Th1) immune responses against cancer-specific antigens. A study showed nanovaccines comprising poly I:C significantly suppress tumor growth in a murine melanoma model without toxicity [181]. Therefore, we believe it would be beneficial to explore how GNP-based vaccines incorporating TLRs as adjuvants could enhance immune responses against melanoma. Thus, we propose further investigation into the potential contributions of GNPs to TLR-based adjuvant vaccines for melanoma immunotherapy.

To sum up, nanovaccines, utilizing nanoparticles for antigen delivery or as adjuvants, offer promising avenues for cancer immunotherapy, particularly in melanoma. GNPs are emerging as valuable carriers for DNA and peptide-based vaccines due to their facile

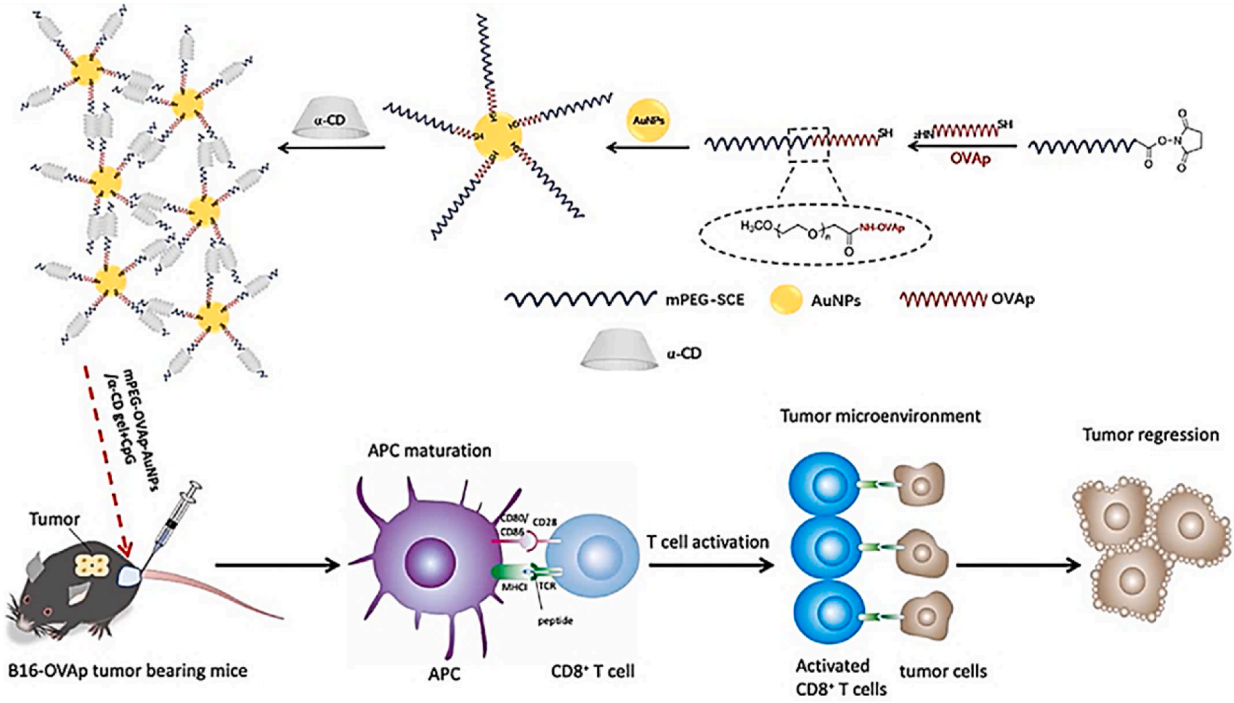

Fig. 6. Injectable nanovaccine for melanoma. Adapted from Xu et al., Materials Today Advances. 2022, 22(4), 1873; DOI: https://doi.org/10.1016/j.mtadv.2022.100236. Licensed under a Creative Commons Attribution 4.0 International License (CC BY). The schematic illustration depicts the development of PEGylated OVA-derived peptide conjugates that bind to gold nanoparticles (AuNPs) through the peptide thiol end-group. These conjugates are combined with α-CD to prepare an injectable hydrogel nanovaccine. This reversible host-guest complex exhibits a long half-life and efficiently encapsulates the peptide, thereby facilitating the delivery. Following injection, the system induces APC maturation and $CD8^+$ T cell activation within the tumor microenvironment, ultimately inhibiting tumor regression [144]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

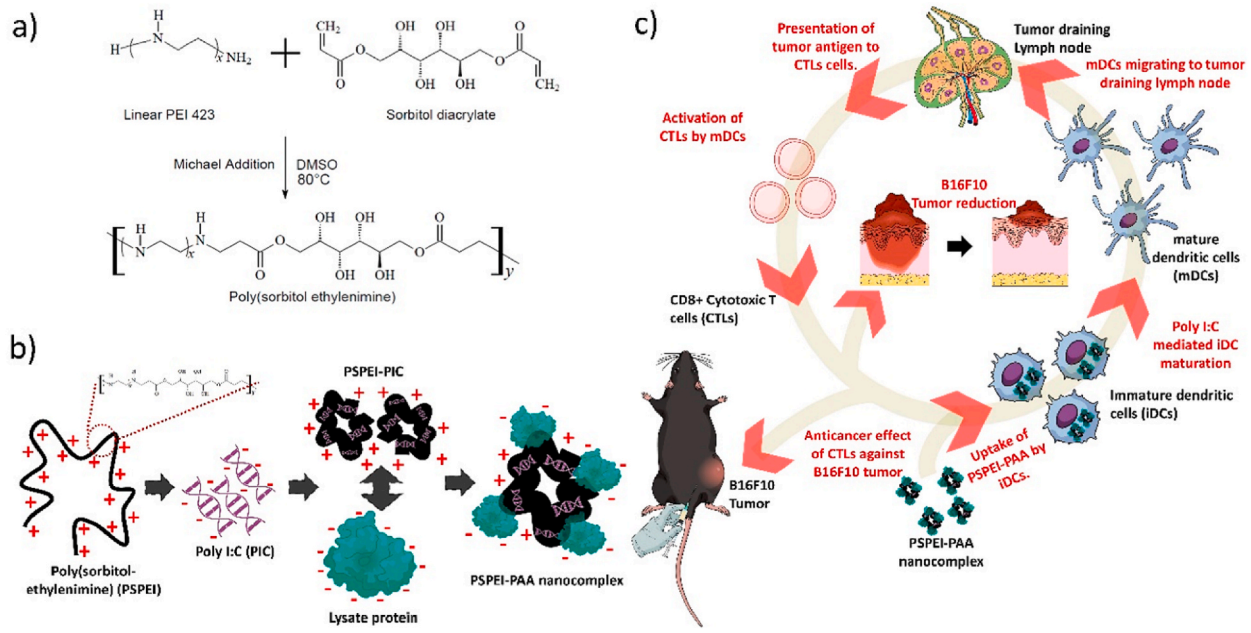

Fig. 7. Self-assembled nanovaccine for melanoma immunotherapy. Adapted from Rajendrakumar et al., Polymers 2018, 10(10), 1063; DOI: https://doi.org/10.3390/polym10101063. Licensed under a Creative Commons Attribution 4.0 International License (CC BY). The poly (sorbitol-ethylenimine) (PSPEI) polyplex antigen/adjuvant (PSPEI-PAA) nanovaccine mediates an anti-tumor immune response against melanoma tumors. (a) Schematic representation of PSPEI synthesized by the Michael addition method, (b) graphic depiction of the formulation of PSPEI-PAA nanocomplex using PSPEI polymer complexed with poly I:C and lysate protein sequentially, and (c) administration of PSPEI-PAA in B16F10 tumors via the peritumoral route initiates the antitumor immune response by maturing dendritic cells and activating cytotoxic T cells. The activated CTLs kill the cancer cells, leading to a reduction in tumor burden [181]. CTL: Cytotoxic T Lymphocyte.

synthesis, functionalization capabilities, and ability to enhance dendritic cell transfection. These nanovaccine formulations address challenges such as rapid antigen clearance and limited immune cell activation within the TME. Furthermore, targeted delivery of adjuvants and antigens utilizing GNPs leads to substantial antitumor immune responses in experimental models. Therefore, integrating GNPs into nanovaccine design holds considerable promise for enhancing vaccine stability, effectiveness, and therapeutic outcomes in melanoma immunotherapy. Finally, we suggest further investigation into Toll-like receptors (TLRs) as adjuvants in GNP-based nanovaccines for melanoma.

5. Imaging application of gold nanostructure in melanoma

In addition to therapeutic applications, gold nanostructures have emerged as invaluable tools in biomarker detection and imaging for melanoma. Due to their tunable size and shape, gold nanostructures can be precisely engineered to interact with specific biomarkers associated with melanoma, providing high sensitivity and selectivity in detection. Moreover, their strong plasmonic properties allow for enhanced signal amplification in various imaging techniques, such as SERS and photoacoustic imaging, enabling the visualization of melanoma biomarkers with exceptional precision. These techniques will be discussed in the following subsections.

5.1. Computerized tomography (CT) and fluorescence imaging

GNPs are highly regarded as promising contrast agents for computerized tomography (CT) due to their remarkable X-ray absorption coefficient, distinctive surface plasmon resonance (SPR) properties, low cytotoxicity, and strong biocompatibility. When conjugated with targeting molecules, GNPs can further enhance imaging capabilities [183,184]. For instance, GNPs have been utilized to label genetically modified T cells expressing a melanoma-specific T cell receptor. These targeted T cells were also engineered to express a green fluorescent protein (GFP) for fluorescence imaging (FLI), creating a dual-labeled system for simultaneous tumor CT and whole-body FLI. The CT scans revealed substantial T-cell accumulation at the tumor sites, and FLI results were found to be in good agreement with the CT findings [145]. Besides, since gold nanoclusters typically exhibit strong fluorescent properties, they can be used as potential contrast agents. In a study, gold nanoclusters conjugated with cyclic arginine-glycine-aspartic acid (RGD) peptide were used for fluorescence imaging of A375 melanoma cells. RGD peptide detects the integrin $\alpha_v\beta_3$, overexpressed in many solid tumors including melanoma. RGD peptide also acts as a protecting and reducing agent, resulting in a rapid, one-step, and inexpensive method for synthesizing gold nanoclusters. Results showed decent stability, biocompatibility, as well as low toxicity, and showed success as contrast agents for FLI in melanoma [98].

5.2. Photoacoustic imaging (PA)

Photoacoustic imaging (PA) is a technique that utilizes non-ionizing laser pulses. When these pulses are absorbed by tissues and converted into heat, they induce transient thermoelastic expansion and the emission of wideband ultrasonic waves. Ultrasonic transducers are then employed to detect these generated ultrasonic waves and create images [184]. Gold nanocages, which were conjugated with [Nle4, D-Phe7]-α-melanocyte-stimulating hormone (NDP-MSH), have found application as contrast agents in PA for melanoma. The results have demonstrated remarkable sensitivity and high specificity, with the conjugated gold nanocages enhancing contrast by approximately 300 % compared to the control group [146]. In a separate study, a versatile polymeric nanoprobe composed of GNRs and liquid perfluorocarbon (perfluorinated hexane or PFH) was linked to a monoclonal antibody of Melanoma-Associated Antigen 1 (MAGE-1 antibody) to target melanoma-associated antigens (MAGE) within B16 cells, effectively serving as a dual-mode contrast agent for PA/ultrasound imaging. The MAGE-1 antibody selectively increased accumulation at the tumor site. Additionally, laser irradiation at 808 nm significantly improved ultrasound imaging. The photothermal effect of GNRs caused the enclosed PFH to vaporize into microbubbles, thereby enhancing photoacoustic imaging resolution [147].

5.3. Surface-enhanced Raman spectroscopy (SERS)

SERS is a vibrational spectroscopy technique that involves amplifying electromagnetic fields and exciting localized surface plasmons. This amplification can detect analytes at low concentrations with high sensitivity [185]. In other words, SERS acts as a highly sensitive and precise Raman fingerprinting method without any interference from background signals [78]. In a study, a planar solid SERS substrate composed of positively charged gold nanoparticles has been utilized to distinguish between melanocytes and melanoma cells. The results demonstrated differences in spectra between various cells with excellent reproducibility in a sample pool of 20, 000 [148]. Given the significance of SPR, noble metal nanoparticles like GNPs are the preferred choice as SERS-active substrates or nanotags. Another critical aspect of SERS involves Raman reporters, materials absorbed on the surface of the SERS-active substrate to induce electromagnetic enhancement. Gold substrates outperform their silver counterparts due to the stronger binding of GNPs with Raman reporters typically containing sulfur or nitrogen atoms in their structure [186]. SERS can also be combined with PCR to enhance the sensitivity of DNA sensing. For example, to specifically identify three clinically significant melanoma point mutations (BRAF V600E, v-Kit L576P, and Neuroblastoma RAS viral oncogene homolog (NRAS) Q61K), PCR-based SERS can detect as few as 10 mutant alleles from a background of 10,000 wild sequences. GNPs were employed as nanotags for SERS, along with various Raman reporters, such as 4-mercaptobenzoic acid for BRAF V600E, 2,7-mercapto-4-methylcoumarin for NRAS Q61K, and 4-mercapto-3-nitrobenzoic acid for c-Kit L576P. The results demonstrated that PCR-based SERS is 100 times more sensitive than SERS alone and can accurately identify mutation carriers with high specificity [149].

5.4. Positron Emission Tomography (PET)

Positron emission tomography (PET) represents a powerful technique for evaluating physiological functions by employing radiolabeled drugs, which offers a quantitative analysis that proves highly beneficial for the continuous monitoring of both disease progression and treatment response. In the context of PET imaging, a radioactive tracer is administered intravenously, and the emitted radiation is meticulously detected, providing valuable insights into the functioning of various biological processes [187]. To illustrate the utility of PET in melanoma research, PEGylated gold nanocages intricately conjugated with NDP-MSH and ^{64}Cu. This precise combination facilitates the sensitive and precise detection of MC1R, a specific melanocortin receptor, within B16/F10 melanoma cells. Such targeted imaging not only aids in early melanoma diagnosis but also offers insights into the expression levels of MC1R, which is vital for understanding the melanoma's behavior and potential treatment options. Furthermore, ^{89}Zr, another element, has been harnessed to label gold nanoparticles (GNPs) for melanoma imaging via PET. This approach has yielded impressive results, as PET scans have consistently demonstrated outstanding tumor contrast in B16F10 melanoma cells [151]. In essence, PET imaging, when combined with advanced nanotechnology and radiolabeling techniques, emerges as a potent tool in the precise and sensitive detection of specific molecular targets within melanoma.

6. Theranostic applications of gold nanostructures in melanoma

Theranostics, a cutting-edge development in nanotechnology and cancer therapy, involves the simultaneous use of treatment and diagnostic components. The groundbreaking advancements in nanotechnology over recent decades have spurred transformative developments across diverse interdisciplinary fields of science, paving the way for the synthesis of increasingly sophisticated, multifunctional, and bioactive materials with promising implications for future research and innovation [188]. Various nanomaterials could be used in theranostics applications such as liposomes, polymeric micelles, quantum dots, nanotubes, dendrimers, iron oxide, solid lipids, and gold nanoparticles [189]. This innovative approach integrates therapeutic and imaging agents into nanoparticles, allowing for the real-time monitoring of various therapeutic aspects, including drug distribution, release, response, and efficacy during administration [15]. For instance, researchers have devised a nanoprobe that incorporates PEGylated gold nanoshells (GNS), cell-penetrating peptides known as TAT, and Cy5-tagged caspase-3 specific peptides (Cy5-DEVD) to create a theranostic platform for regulating PTT and detecting apoptosis in B16F10 melanoma. The nanoprobe exhibits a highly effective photothermal capability, wherein GNS, when irradiated with a laser, can cleave the Cy5-DEVD peptide, resulting in a remarkably sensitive fluorescent signal. Consequently, when injected into a mouse model, the tumor area exhibits an increasing fluorescent signal over time. This feedback

from caspase-3 imaging enables the adjustment of the photothermal dose accordingly [152].

In another study, GNPs attached to an anti-Ganglioside D2 (anti-GD2) antibody for PTT were combined with single-wall carbon nanotubes (SWCNT) to create a hybrid theranostic platform capable of selective imaging upon exposure to the second NIR light. This innovative approach was tested on melanoma UACC903 cells and yielded striking results. Following just 8 min of NIR irradiation, nearly 100 % of melanoma cells were rendered nonviable. In contrast, when anti-GD2-GNP or anti-GD2-SWCNT were used alone, less than 10 % of the cells were eliminated. In this context, the anti-GD2-attached theranostic material serves a dual purpose as a photothermal agent, through radioactive decay, and as an enhancer of optical energy absorption, akin to a local nanoantenna, facilitating selective two-photon imaging. Furthermore, it was demonstrated that two-photon luminescence signals remained nearly unchanged even after an hour of exposure at 775 nm [153]. In another innovative theranostic platform, SERS was employed to monitor the behavior of the chemotherapy drug dabrafenib. Dabrafenib was covalently attached to GNPs that had been functionalized with folic acid and transferrin, enabling precise targeting of folate and transferrin receptors in melanoma. The SERS technique confirmed the controlled release of dabrafenib via folate receptors, offering a promising avenue for improved drug delivery and melanoma treatment [154]. In a separate study, a theranostic approach utilized liposomal layer-coated gold nanocages modified with an antibody specific to dendritic cells, aCD11c. These nanocages were engineered to deliver the adjuvant monophosphoryl lipid A (MPLA) and melanoma antigen peptide TRP2, effectively functioning as a nanovaccine targeting B16F10 melanoma cells. The process also integrated fluorescence and PA imaging to monitor the immune response. This innovative nanoprobe exhibited the ability to efficiently inhibit tumor growth by enhancing the immune response, showcasing its potential in melanoma therapy [85]. Yet another versatile theranostic platform involved liposomal layer-coated gold nanocages, creating a nanoprobe in which the GNCs were labeled with 4-mercaptobenzoic acid, coated with a liposomal layer, and finally decorated with an anti-Melanoma Cell Adhesion Molecule 18 (anti-MUC18 single-chain antibody) (Fig. 8). SERS mapping was employed to selectively identify A375 melanoma cells, and PTT effectively eliminated these cells without significant side effects. The results demonstrated high biocompatibility, the potential for selective melanoma diagnosis, and remote-controlled PTT, highlighting the versatility and promise of this theranostic platform [78].

As discussed earlier in section 4, a nanoprobe composed of GNRs and PFH was conjugated to a monoclonal antibody (MAGE-1 antibody) to facilitate PTT and optical droplet vaporization (ODV) therapy, which was monitored using ultrasound, specifically contrast-enhanced ultrasound (CEUS), in the B16F10 mouse melanoma cell line. The combination of the photothermal effect and the physical damage induced by ODV significantly improved tumor ablation compared to the observed tumor recurrence when PTT was employed alone. Additionally, laser irradiation was shortened, minimizing damage to surrounding healthy tissue. Furthermore,

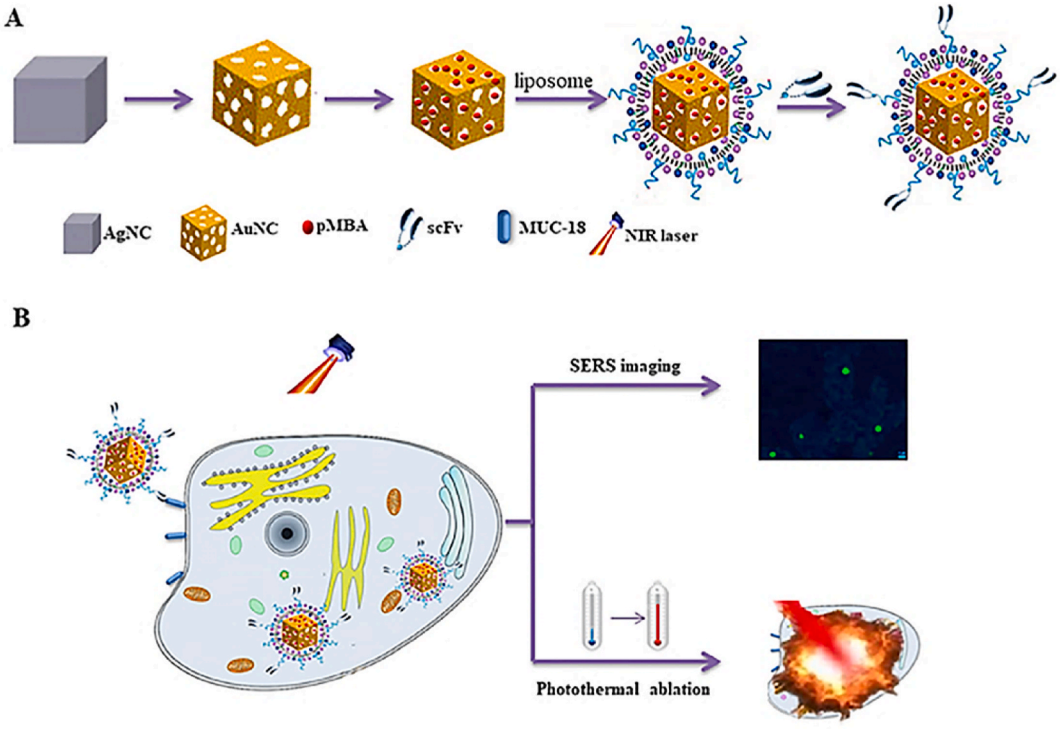

Fig. 8. A schematic illustration depicts the preparation and theranostic capabilities of liposomal layer-coated gold nanocages (AuNCs) decorated with anti-MUC 18 single-chain variable fragment (scFv). These AuNCs are prepared by coating pMBA (para mercaptobenzoic acid)-laden AuNCs with phospholipids and then incorporating anti-MUC 18 scFv-PEG-lipids using the post-insertion method. The system has been utilized for SERS imaging and inducing photothermal ablation in melanoma cells. The liposomal coating prevents Raman tag leakage and improves biocompatibility. The high cellular binding/uptake results in improved photothermal effects [78]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

ultrasound imaging was greatly enhanced at the tumor site due to laser irradiation (Fig. 9). Notably, CEUS imaging showed a gradual weakening with each treatment repetition, suggesting the viability of this technique for monitoring the effectiveness of tumor ablation [190].

An exceptional case encompasses a three-in-one theranostic platform that combines chemotherapy with PTT and photodynamic therapy PDT, utilizes a monoclonal antibody for targeted delivery, and monitors the process using SERS. The foundation of this platform consisted of GNRs with bovine serum albumin (BSA)-stabilized gold nanoclusters enveloped on their surface, serving as a SERS substrate. Dacarbazine (DAC), a chemotherapy drug, was loaded onto this base platform alongside a novel squaraine molecule called ISQ, which served as a photosensitizer and a Raman reporter for DAC. Finally, the nanoenvelopes (Fig. 10) were conjugated with anti-Death Receptor 5 (anti-DR5) to specifically target A375 melanoma cells. This synergistic approach, combining laser-induced PTT and PDT with chemotherapy, effectively induced apoptosis in melanoma cells, as confirmed through SERS analysis [155].

In summary, theranostics represents an exciting frontier in cancer therapy. These approaches allow for real-time monitoring and adjustment of therapeutic interventions, potentially revolutionizing the precision and effectiveness of melanoma therapy.

7. Safety and potential toxicity of gold nanostructures

Despite the considerable interest in GNPs and their potential, some major challenges limit their clinical uses. A particularly

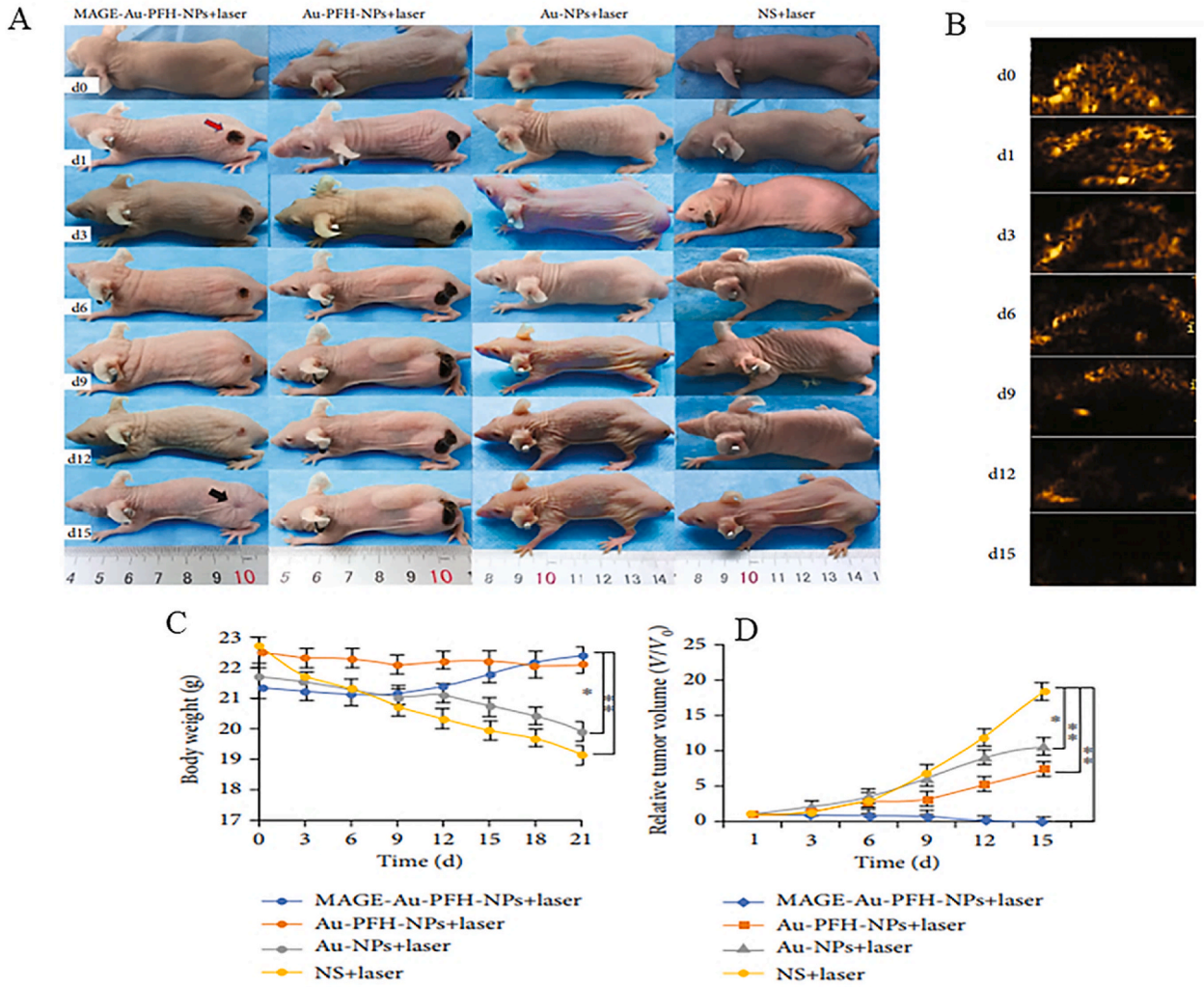

Fig. 9. Assessment of photothermal and optical droplet vaporization (ODV) capability of the MAGE-1 antibody-conjugated Au-perfluorohexane nanoprobe (MAGEAu-PFH-NP). Adapted from Li et al., BioMed Research International 2020; DOI: https://doi.org/10.1155/2020/6863231. Licensed under a Creative Commons Attribution 4.0 International License (CC BY). (A) The images show B16 tumor-bearing mice treated with Au NP, Au-PFH-NP, MAGEAu-PFH-NP, or normal saline (NS) combined with laser irradiation. (B) Ultrasound images capture changes in the region of interest in B16 tumor-bearing nude mice over 15 days following laser irradiation. (C and D) Plots display variations in body weights and tumor volumes among the different treatment groups [143].

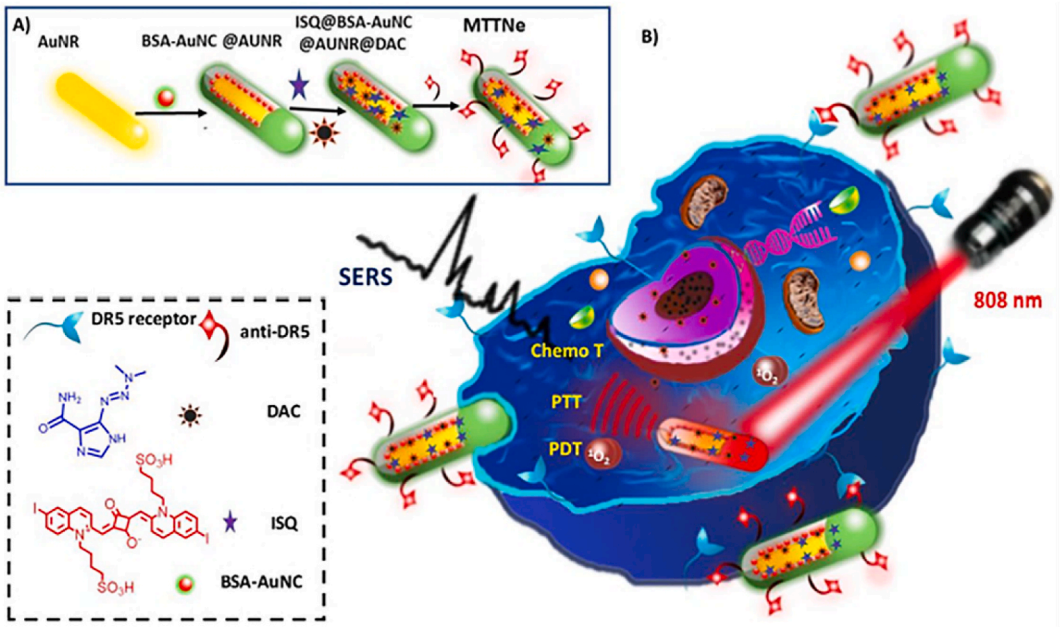

Fig. 10. Schematic illustration of (A) the preparation steps for the melanoma-targeted theranostic nanoenvelope (MTTNe): coating of BSA-stabilized gold nanoclusters (BSA-AuNCs) onto gold nanorods (AuNR), loading of squaraine (ISQ) and dacarbazine (DAC), and conjugation with anti-DR5. (B) Demonstrating the theranostic capabilities of MTTNe for targeted SERS imaging and combinational therapy (PTT/PDT/Chemo) of melanoma cancer cells [155]. This strategy improves not only biocompatibility and stability but also photothermal conversion and SERS enhancement efficiency simultaneously. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

significant setback in medicine is the unclear toxicity profile of GNPs *in vivo* [75]. *In vitro* studies have demonstrated cytotoxicity through various mechanisms, including oxidative stress, inflammatory response, long-term DNA damage, protein denaturation, etc., depending on the nanoparticles' shape, size, and surface charge [191,192]. Balancing the bioactivity and therapeutic properties of GNPs while minimizing cytotoxicity could be a challenge. For instance, spherical GNPs exhibit lower toxicity but also have limited potential due to poor photothermal conversion efficacy [193]. However, another study found gold nanospheres and nanorods more toxic than other shapes such as stars or flowers [75]. Unfortunately, the current *in vivo* results are insufficient, and often contradictory, emphasizing the urgent need for more diverse and long-term *in vivo* and *in vitro* studies. While *in vitro* data are valuable, they can never replace *in vivo* results, underscoring the importance of understanding the toxicity profile of GNPs in various animal species. In one study, mice died due to a loss of appetite after exposure to 8–37 nm colloidal GNPs [191]. As mentioned earlier, a positive aspect of GNPs is their ease of functionalization and incorporation of molecules to improve their bioactivity and toxicity. For instance, coating gold nanorods with the negatively charged gum-arabic reduces the toxicity of the positively charged surfactant, CTAB, toward normal fibroblast cells by 30 % [194]. Furthermore, a comparative study showed that PEG-coated GNPs induced less hepatotoxicity than uncoated GNPs in Sprague Dawley rats, suggesting that PEG-coated GNPs might offer a safer option for biomedical applications [195]. However, the challenge lies in testing each of these ligands, capping agents, stabilizers, etc., *in vivo*. For instance, in mice, citrate-capped GNPs exhibit significant toxicity, whereas GSH-coated GNPs show no toxicity. Moreover, the type of drug co-administered with GNPs could also influence the toxicity profile. For instance, GNPs display nephrotoxicity when administered with cisplatin, paraquat, and 5-aminosalicylic acid [75].

Regarding possible accumulations, studies have revealed traces of GNPs in the brain, lungs, heart, and evidence of sperm toxicity, but no long-term toxicity study has been conducted yet [196]. While ongoing clinical trials for treating other diseases have shown no major toxicity, it's important to note that these trials are relatively short-term and have small sample sizes. Consequently, larger, more long-term studies are needed to understand the potential effects of gold accumulation in various organs [120].

8. Conclusion and future perspectives

Metastatic melanoma has long posed significant challenges in both diagnosis and treatment. However, the promising application of nanotechnology, particularly the utilization of gold nanostructures, has emerged as a beacon of hope in addressing these challenges. Gold nanostructures, varying in size and morphology, exhibit physicochemical properties that make them highly suitable for numerous applications. These properties include ease of functionalization, remarkable biocompatibility, small size, and exceptional optical characteristics, particularly surface plasmon resonance (SPR). By exploring various therapeutic modalities such as photothermal therapy, photodynamic therapy, and targeted drug and gene delivery, GNPs demonstrate remarkable potential in combating this

aggressive form of skin cancer. Additionally, GNPs have played a pivotal role in the development of novel melanoma vaccines, aiming to trigger robust immune responses against cancer cells. Moreover, GNP integration into melanoma imaging and theranostic applications further underscores their versatility and significance in clinical practice. Using modalities like CT, fluorescence imaging, PA, SERS, and PET, GNPs facilitate precise diagnosis, enabling early detection and accurate staging of melanoma, thereby informing treatment decisions and enhancing patient outcomes. Most significantly, the combination of treatment and diagnosis, known as theranostics, has been greatly advanced by applying gold nanostructures. This integrated approach not only enables real-time monitoring of therapeutic efficacy but also facilitates the development of personalized treatment strategies.

While gold nanostructures show significant promise, several obstacles and challenges should be addressed to fully harness their potential. Traditional methods in GNP synthesis using hazardous chemicals pose risks to health and the environment. However, green synthesis offers a promising alternative. By utilizing plant extracts like green tea, aloe vera, and geranium leaf as natural reducing agents, or plant-based materials such as phytochemicals from cinnamon or mangoes, we embrace eco-friendliness, renewable sources, and pollution reduction. This approach can also be cost-effective and yield biocompatible nanoparticles for biomedical use. Green synthesis aligns with sustainability principles, offering a transformative pathway to a more ethical future in nanotechnology [197, 198]. In addition, certain natural sources exhibit multifunctionality. For instance, alfalfa sprouts not only accumulate gold ions but also serve as reducing and stabilizing agents in GNP synthesis in GNP synthesis. Fungi, such as Fusarium oxysporum and Aspergillus fumigatus, demonstrate promise as their extracellular enzymes and metabolites effectively serve as reducing and stabilizing agents in nanoparticle synthesis [198].

Scalability poses a significant limitation to gold nanostructures in melanoma diagnosis and treatment due to the time-consuming and expensive production process, potentially restricting their clinical application. Large-scale production of nano-based products encounters stability and reproducibility challenges stemming from batch-to-batch differences, emphasizing the need for uniform definitions and standards to ensure a consistent pharmacokinetic profile.

Biocompatibility evaluation is crucial to prevent adverse effects on healthy tissues and organs, while long-term toxicity and potential accumulation in the body require thorough assessment. Achieving precise control over the size and distribution of gold nanostructures is essential for consistent performance yet remains challenging. Targeting melanoma cells specifically without affecting healthy cells remains a hurdle for functionalized gold nanoparticles used in drug delivery. Strategies to evade or modulate the immune response to increase circulation and retention of gold nanostructures at the tumor site are critical. Optimizing drug-loading strategies and release kinetics is imperative for effective treatment, necessitating further research and development efforts.

GNPs are under investigation in clinical trials examining their phototherapeutic potential, including drug delivery for breast cancer and the application of gold nanoshells in photothermal therapy for prostate, head, neck, and lung cancers [199]. Failures in clinical trials within drug development, including nanomedicines, often arise due to unanticipated effectiveness issues and safety concerns not foreseen during preclinical studies. A survey of cancer nanomedicines highlighted a significant decline in success rates from Phase I (94 %) to Phase II (48 %) and Phase III (14 %). This underscores the challenge of translating promising early-stage results into clinical success. Additionally, the understanding of the life cycle of GNPs within the body remains incomplete, including uncertainties surrounding their fate post-cellular internalization [200]. These knowledge gaps underscore the need for further research to clarify the behavior and potential risks of GNPs, which are crucial for their successful clinical translation.

A new approach for evaluating toxicity in nanomedicine is the use of artificial intelligence (AI). AI has already proven its potential in tumor detection, predicting therapeutic response, and toxicity [201]. AI has shown promise in clinical dermatoscopy and pathological slide assessment in diagnosing and managing cutaneous melanoma, particularly for patients with advanced stages of the disease [202]. AI-based approaches can analyze extensive drug libraries to determine optimal dosages and supply precise drug doses [203]. These approaches could also optimize combination therapy by selecting drugs with the best synergy and the least toxicity, as well as improving drug targeting and personalized dosing [204]. Machine learning is a branch of AI that predicts future outcomes based on previous data. It can recognize complex patterns and compute large datasets [201]. AI techniques have been shown to help determine GNP cytotoxicity, protein bindings, and even possible DNA interactions [201]. Furthermore, melanoma cells can develop resistance to therapies over time. Developing strategies to overcome or delay resistance is a key challenge in melanoma treatment using gold nanostructures. Clinical trials investigating various compositions of GNP-based systems have shown promising safety profiles across different administration routes. Reported adverse events were transient, clinically manageable, or unrelated to GNP treatment. However, limitations include the small sample sizes and sparse trials, with many studies evaluating only one therapeutic dose [205]. Quality improvement is needed, as some trials were not registered in national databases or published. A notable finding from one study revealed gold content in tumors even 174 days post-treatment, suggesting the need for further investigation into potential long-term side effects of gold accumulation. Multi-dose, multi-center blinded trials have been initiated to address these gaps and expand clinical understanding of GNPs [198].

Besides, the regulatory approval for using gold nanostructures in cancer diagnosis and treatment is still in the early stages. The lack of standardization can lead to variations in the properties and performance of these structures, which can affect their safety and efficacy. The regulatory process for new drug or therapy approval can be complex and time-consuming, requiring extensive testing and validation.

In conclusion, the diverse applications of gold nanostructures in melanoma research and therapy hold great promise. Their unique properties enable us to address the complexities of metastatic melanoma, advancing both diagnosis and treatment. Continued research and innovation are crucial for integrating gold nanostructures into clinical practice, potentially revolutionizing our approach to combating this challenging cancer. However, transitioning from preclinical studies to clinical trials and eventual clinical use requires rigorous safety and efficacy assessments, regulatory approvals, and large-scale production of standardized nanostructures. Moving forward, interdisciplinary collaborations and concerted efforts in translational research are essential for harnessing the full therapeutic

potential of GNPs and improving outcomes for patients affected by melanoma.

Here are some ideas for overcoming the current challenges:

Firstly, addressing the limitations associated with animal models used in melanoma research is crucial. Despite advancements in finding potentially better animal models for melanoma, such as zebrafish or patient-derived xenograft models, the predominant use of mice and rats persists in preclinical studies. There is an urgent need for more extensive investigation into alternative animal models that better mimic the complexities of human melanoma. Research should focus on identifying and developing animal models that accurately represent the diverse subtypes and stages of melanoma, thus enhancing the translational relevance of preclinical findings.

Secondly, efforts need to be directed towards researching GNPs in melanoma more efficiently, and AI presents a promising avenue for achieving this goal. Establishing a specific AI engine dedicated to GNP research could significantly enhance the efficiency of studies in this field. By uploading previous and upcoming research findings into the AI engine, we can expand the data pool and enable AI to accurately predict factors such as effectiveness, toxicity, and other crucial parameters. This approach would streamline research processes, saving both time and resources. Additionally, incorporating cost-benefit analyses into research protocols can provide valuable insights into the feasibility and practicality of GNP-based interventions.

Furthermore, personalized medicine approaches, such as genetic profiling and biomarker analysis, tailor treatment to each patient's genetic profile and disease attributes, optimizing effectiveness while minimizing side effects. GNPs can be customized with nucleic acid probes or antibodies to identify specific genetic mutations or biomarkers linked to melanoma. This enables clinicians to tailor therapy based on tumor molecular characteristics, enhancing treatment precision. Additionally, coupling GNPs with imaging agents, such as fluorescent dyes or contrast agents, allows clinicians to monitor treatment response and make personalized therapy adjustments. GNPs can also enhance the effectiveness of radiation therapy as radiosensitizers. Integrating GNPs into radiation therapy enables personalized radiation dosing tailored to individual tumor features and radiosensitivity.

Finally, we propose further exploration of hybrid GNPs in melanoma research. Hybrid nanoparticles have the potential to mitigate the potential toxicities associated with gold while adding further functional properties. By using reduced amounts of gold in hybrid formulations, researchers can achieve comparable therapeutic efficacy while minimizing the economic and environmental burdens associated with GNPs.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

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CRediT authorship contribution statement

Zahra Sadat Dastgheib: Writing – original draft. **Samira Sadat Abolmaali:** Writing – review & editing, Investigation, Conceptualization. **Ghazal Farahavar:** Writing – review & editing, Conceptualization. **Mohsen Salmanpour:** Writing – review & editing. **Ali Mohammad Tamaddon:** Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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