Nanomedicine in Melanoma: Current Trends and Future Perspectives

AYMAN EL-MEGHAWRY EL-KENAWY1,2,* • CAROLINA CONSTANTIN3,4 • SNUR M. A. HASSAN5 • ALSHIMAA MOHAMED MOSTAFA6 • ADRIANA FREITAS NEVES7 • THAISE GONÇALVES DE ARAÚJO8 • MONICA NEAGU3,4,9

1Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Sadat, Egypt; 2Department of Pathology, College of Medicine, Taif University, Taif, Saudi Arabia; 3Immunology Department, Victor Babes National Institute of Pathology, Bucharest, Romania; 4Colentina University Hospital, Bucharest, Romania; 5Department of Anatomy and Pathology, College of Veterinary Medicine, Sulaimani University, Sulaymaniyah, Kurdistan-Iraq; 6Department of Dermatology, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt; 7Molecular Biology Laboratory, Institute of Biotechnology, Universidade Federal de Goias, Catalao, Brazil; 8Laboratory of Genetics and Biotechnology, Institute of Genetics and Biochemistry, Federal University of Uberlandia, Patos de Minas, Brazil; 9Faculty of Biology, University of Bucharest, Bucharest, Romania

Author for correspondence: Ayman El-Meghawry El-Kenawy, Department of Pathology, College of Medicine, Taif University, Saudi Arabia. Email: elkenawyay@yahoo.com

Doi: http://dx.doi.org/10.15586/codon.cutaneousmelanoma.2017.ch10

Abstract: As cutaneous melanoma is a highly aggressive and drug-resistant cancer, there is intense research focusing on developing new, efficient drugs. Nanomedicine focuses on developing different groups of nanomaterials for both diagnosis and therapy, and this combination of specific diagnosis and therapy is called theranostics. Nanomaterials tailored as delivery vehicles can be nanocapsules, nanorods,
nanotubes, nanoshells, and nanocages. All these structures protect the intended drug against degradation and enhance its stability. The development and characterization of polymeric nanoparticles, polymeric micelles, liposomes, nanohydrogel, dendrimers, inorganic nanoparticles, and hybrid nanocarriers are among the delivery vehicles that transport different anticancer agents. Functionalization of nanocarriers with specific molecules, such as antibodies, can generate different smart nanodrugs for application in cancer therapy and/or diagnosis. Nanotherapeutic strategies deal with several shortcomings that comprise of tumor characteristics, biological barriers, biocompatibility, and so on. As nanostructures interact with various host biomolecules, comprehensive in vitro cellular models call for evaluation of physicochemical properties, dose, and time of action of nanomaterials, while in vivo assessments would provide valuable data regarding the level of absorption, tissue/organ distribution, and metabolism. The future perspectives in nanotechnology applied to cancer overcomes the translational barrier from the laboratory to the clinical application to potentially improve conventional theranostic techniques.

**Key words:** Melanoma; Nanomedicine; Nanotechnology; Theranostic; Treatment

### Introduction

Melanoma, the cancer of melanocytes, is the sixth most frequently diagnosed cancer in humans and accounts for 80% of skin cancer–related deaths (1). Morbidity and mortality indices are highly variable worldwide—being rare in nations of Asian and African origin and almost considered epidemic in countries of Caucasian predominance (2, 3). When diagnosed early, as a localized cutaneous tumor, melanoma can be surgically removed with a good prognosis (4). Once melanoma becomes metastatic, it turns into a more aggressive and difficult to treat malignancy (5). Management of metastatic melanoma is challenging if the tumor becomes unresectable or if it recurs shortly after resection (6). In such cases, other conventional treatment options including chemotherapy, radiotherapy, targeted therapy, and photodynamic and immunotherapy have to be combined with surgery (7).

Dacarbazine (DTIC) is the first chemotherapeutic treatment approved by U.S. Food and Drug Administration (FDA) for metastatic melanoma (8). Temozolomide, a DTIC derivative, has the ability to cross the blood–brain barrier and is a first-line therapy for brain metastases (9). Recently, BRAF inhibitors (Vemurafenib, Dabrafenib) and MEK inhibitor (Trametinib) have been approved by the FDA for treating BRAF-mutated melanoma which is nearly found in 50% of cases (2). Immunotherapy is another promising treatment option in metastatic melanoma. Ipilimumab, an anti-cytotoxic T-lymphocyte antigen 4 antibody (CTLA-4), and nivolumab and pembrolizumab, programmed death receptor 1 (PD-1) inhibitors, have been approved for use in the treatment of metastatic melanoma (10, 11). However, despite these recent therapeutic breakthroughs, there are still some drawbacks including undesirable side effects, tumor chemoresistance, or even disease relapse (2, 12). As cutaneous melanoma is a highly aggressive cancer (13), there is intense research focus on developing new, efficient drugs. Taken together,
these challenges have led researchers to explore new ways of early diagnosis and investigate novel approaches of drug delivery to reach high efficacy, minimal toxicity, and less failure—advantages that melanoma-related nanotechnology could potentially offer (14, 15).

Nanomedicine for Melanoma Detection and Treatment

Early diagnosis of melanoma is essential to increase patients’ survival rates. The 10-year survival rate for Stage IA is 93%, while patients diagnosed at Stage IV have a 10-year survival rate of 10–15% only (16–18). Moreover, the cost of treating melanoma increases dramatically with later stages of the disease (19–21). In addition to the clinical and histological examination, many new techniques have been utilized to aid early detection of melanoma. These techniques include dermoscopy, total body photography, multispectral digital imaging analysis, and RNA microarray (22, 23). In-depth investigations of the molecular changes of metastatic melanoma have paved the way for more advanced technologies known as molecular diagnostics. They include fluorescent in situ hybridization (FISH), next-generation sequencing (NGS), quantitative reverse transcription-polymerase chain reaction (qRT-PCR), comparative genome hybridization (CGH), and detection of exosomes (24–27). Nanotechnology is one of the promising tools recently used for detection of melanoma with high sensitivity and specificity (28–30).

Nanoparticle quantum dots (QDs), fluorescent nanoparticles characterized by excellent brightness, narrow field of emissions, broad absorption spectrum, and excellent photostability, have been suggested as a useful technique for cancer detection (31–35). Those photophysical properties allowed researchers to conjugate QDs with variable cancer-specific molecules as folic acid or antibodies against specific cancer antigens (36–40). When QDs are conjugated with specific anti-melanoma antibodies (e.g., HMB45, MART-1, and Tyrosinase), melanoma cells can be distinguished from normal melanocytes (41). However, the heavy metal composition of QDs, with its high toxicity and immunogenicity, hinders the wide application of QDs as an imaging modality for cancer (42, 43). Recently, coating QDs with a polyethylene glycol (PEG) have been shown to decrease cytotoxicity (44). Similarly, Cornell dots known as C-Dots are PEG-coated silica-based nanoparticles that are used as probes to guide sentinel lymph node biopsy (SLNB) (45, 46). These FDA-approved nanoparticles are used as PET-optical or optical probes that particularly target RGD peptides attached to alpha 2 beta 3 integrin overexpressed in melanoma cells (47–50). Nanotechnology has been used in medicine for developing nanometer scale materials, ranging from 1 to 100 nm, having therapeutic and diagnostic purposes (51–53). Nanomaterials’ size range matches cellular organelles, other molecules involved in intracellular events, as signaling pathways, and/or molecules involved in cell to cell communication (16, 20). The nanomaterials bio-distribution is dependent on the surface charge, biodegradability, size, their distinct biological properties, and shape (19, 21–25). Nanoparticles (NPs) or nanocapsules are the most common shape for nanomaterials used as drug delivery systems. Moreover, this shape offers protection against degradation, enhances its stability, driving an efficient accumulation at target sites (26).
Currently, nanorods, nanotubes, nanoshells, and nanocages are nanomaterials with imaging and cancer therapy applications (26, 27).

Carbon-based nanoparticles are effective in melanoma cells (53). Thus, a single-walled carbon nanotube loaded with DOX-induced melanoma cell death in a dose-dependent manner in vitro and revoked tumor development in a xenograft melanoma model. Gold nanoparticles (GNPs) are known as nontoxic, highly stable, easy to synthesize, and minimally interfering with the biological profile of melanoma tumor cell (54, 55). Being of high atomic number and electron density, GNPs are optimal contrast agents for computed tomography (CT) (56, 57). When labeled with radioisotope indium-111 and conjugated with RGD ligands, GNPs were successfully used as radiotracers in experimental melanoma models (58). Meir et al. have shown in melanoma-bearing mice that labeled GNPs can track tumor-specific T-cells using whole body CT. This approach is a next-generation imaging technique as well as a new tool in immunotherapy (59).

Magnetic nanoparticles (MNPs) were successfully used in MRI (60). In the recent MELAMAG clinical trial, SLNB detection based on MNPs was compared to the standard technique. In this study, MNPs with small iron nanoparticles (named Sienna+ by the developers) were intradermally injected and a hand-held magnetometer was used intraoperatively to detect the accumulation of MNPs. A gamma probe was used as comparator and the results showed the feasibility of the magnetic technique for SLNB detection. The highest identification was proven for inguinal and axillary lymph nodes, while the lowest detection rates were registered for the cervical region. From 129 recruited patients, the study reported 95.3% rate of sentinel node identification using this MNPs-based technique (60).

Another nanoparticle tested for contrast-enhanced MRI lymphography is Gadolinium-loaded nanoparticles (Gd-FVT). Using these NPs, the specificity and sensitivity of MRI lymphography in melanoma-bearing mice could be enhanced (61).

Zhou et al. developed an efficient and noninvasive strategy to detect melanoma metastasis in LN using Gd-embedded iron oxide nanoplates (GdIOP), functionalized with Zwitterionic Dopamine Sulfonate (ZDS) molecules. With T1-T2 dual-modal MRI, GdIOP@ZDS nanoparticles were highly taken up by dendritic cells and macrophages in LN, in contrast to melanoma B16 tumor cells which showed lower uptake. This generated difference represented pseudo-contrast images which can be potentially used for detection of melanoma metastasis in LN (62).

RGD-targeted nanoparticles of iron oxide (NPIO) were previously utilized for MRI of in vivo tumor angiogenesis with variable limitations including long blood half-life and nonspecific extravasation (63). Nevertheless, conjugation of cyclic RGD variant [c (RGDyK)], with enhanced affinity for αvβ3, a specific marker of angiogenesis, to iron oxide microparticles (MPIO) provided a more sensitive molecular MRI approach (64).

Another promising application of nanotechnology is the detection and quantification of circulating tumor cells (CTCs) as a blood-based biomarker “liquid biopsy” (65). Seenivasan et al. immobilized anti-Melanocortin 1 receptor antibodies (MC1R-Abs) on amino-functionalized silica nanoparticles (n-SiNPs)-polypyrrole (PPy) nanocomposite thin film and used them as an immune sensor
for selective and sensitive detection of melanoma cells (66). A magnetite nanoparticle designed by Sato et al. by conjugating N-propionyl-cyst aminyl phenol with magnetite was used in a B16F1 xenograft mouse model (67). Souza et al. showed that melanoma cells were degraded after the application of an external irregular magnetic field to increase the temperature in the tumor to 43°C. The nanoparticle had a 1.7- to 5.4-fold greater effect compared with magnetite alone (46).

### Nano Therapies: Radiotherapy and Chemotherapy

NPs in the context of radiotherapy and chemotherapy are particularly interesting. Radiotherapy and surgery are local treatments, while the main systemic strategy is chemotherapy, especially considering the risk of metastasis (30). Radiotherapy has a limited role in treating melanoma patients and is used selectively. Its success is limited due to radiation resistance in melanoma cells (16, 34). This technique has been improved by engineering, physics, chemistry, and biology to promote innovative technologies that allow real-time imaging and better dose distribution according to disease progress (67).

In general, the radioisotopes used in medicine emit energy that produces DNA cleavage, damage that is induced mainly by ionized atoms and free radicals. The clearance performed by the kidney is dependent on the size of the radioisotopes. Molecules smaller than 5 nm are excreted rapidly and fail in promoting desirable effects due to short circulation time in blood. Immune response, including opsonization, is another way for radioisotopes clearance by mononuclear phagocytes (MPS). In this context, nanocarriers emerge as an alternative for the half-life increment of radioisotopes (67). Glutathione-coated gold nanostructures represents the next generation of radiosensitizers used for gamma-ray irradiation (34, 68). Moreover, PEGylation of NPs produces nanocarriers that prevent opsonization, increasing the half-life of the radioisotopes (69). Carbon nanostructures have also been related as potential nanocarriers used in radiotherapy, displaying particular physicochemical properties (70) as ultralight, conductivity, and high-surface area (71).

### POLYMERIC NANOPARTICLES

Polymeric nanoparticles (PNs) are molecules usually organized with tunable size into a dense structure with entangling biodegradable polymers presenting thermodynamic stability in an aqueous solvent (72–75). Recently, FDA approved three PNs, namely, polylactic acid (PLA), poly (lactic-co-glycolic acid) 43 (PLGA), and polycaprolactone (PCL). The hydrophilicity for the encapsulation of hydrophilic drugs is one of the deficiencies for the desired release of the encapsulated agents (32). Copolymers as polyethylene glycol (PEG)ylated have been used to reduce the degradation rate of PN to produce PLA-PEG, PLGA-PEG, improving their biocompatibility and modifying its amphiphilicity. Furthermore, PEG has been described as a strategy to evade immune response (76, 77).
LIPOSOMES AND NIOSOMES

Liposomes can remain in the blood circulation longer, permitting continued drug release with increased precision in tumor-targeting (78–84). They can incorporate nucleic acids and other organic or inorganic molecules into their aqueous lumen (85–89) and can be used for targeted, controlled drug release (90–96).

Thus, in two melanoma xenograft models, phosphatidylethanolamine liposomal cisplatin was proven to have higher cytotoxicity than classic liposomes or free cisplatin, a high concentration of intratumoral drug remaining for 72 h and efficiently delivering 3.6-times more drug compared to the free drug (97–103). Niosomes are biodegradable, biocompatible, nontoxic, and nonimmunogenic having extensive solubility and flexibility. Niosomes have been confirmed to have prolonged circulation, increased drug retention in skin, and enhanced drug spreading when topically applied (104–107). Dwivedi et al. proved that encapsulated artemisone which is a 10-amino-artemisinin derivative with antitumor activity in niosomes exhibited extremely selective cytotoxicity toward the melanoma cells but not to the normal skin cells (108).

NANOHYDROGELS

Nanohydrogels are cross-linked hydrophilic soft polymers organized in a tridimensional network comprising a large fraction of water (28, 32). The nanohydrogels’ cross-linking occurs through hydrophilic–hydrophobic interactions, hydrogen bonds, electrostatic interactions, or covalent bonds. The aqueous environment promotes the swelling of nanohydrogels, a characteristic that is determined by the degree of the cross-linking and external environment. This nanocarrier is promising for multimodality treatment, especially for peptides, proteins, and oligonucleotides, because of their hydrophilicity and efficient cell uptake. The co-delivery of PTX and DOX drugs in nanogels are possible due to the positively charged surface that could load negatively charged proteins (32). Functionalized nanohydrogels siRNA delivery systems that target epidermal growth factor receptor were tested in an ovarian cancer mouse model in a platinum-based therapy (82). Polymersome could be valuable for melanoma treatment owing to its benefits, such as robustness, increased drug loading, constancy, relatively longer in vivo circulation, and the possibility to design it for the delivery of multiple drugs (104). Polymersomes have been used to carry DOX for melanoma therapy and established to be specially taken up by melanoma cells (109–111).

THERANOSTIC NANOMEDICINE AND MELANOMA THERAPY

By using nanoparticles for both diagnosis and treatment, theranostic nanomedicine has been advanced recently (112, 113). Liposomes, exosomes, polymersomes, nanocrystals, nanotubes, and nanowires are among the commonly used nanoparticles and nanodevices, and endless combinations can be created with these nanostructures (114). Some metals, such as gold (Au) and Gadolinium (Gd), can have antitumor activity besides being an imaging tracer (88). Gd-based NPs (AGuIX) were successfully used as both MRI contrast agent and therapy in
experimental animal models of melanoma metastases (115–120). Another novel theranostic nanostructure for melanoma was a NP biodegradable photoluminescent polymer—poly (lactic acid) (BPLP-PLA) loaded with anti-BRAF V600E-specific drug (PLX4032) and muramyl peptide. The new immune-cell-mediated nanoparticle offers high hopes for melanoma imaging and treatment (121–126).

**Current Limitations and Exploring Possibilities for Improving the Efficiency of Nanodrugs in Melanoma**

Although notable progress has been made in the synthesis and characterization of nanodrugs, and we are witnessing the first clinical trials that have shown promise (127–130), there are still limitations that should be overcome. Thus, nanodrugs, once having entered the biological system, complexly interact with the host’s immune system, leading to premature clearance, side-effect activation, and toxicity (131–135). Consequently, the main limitations of nanodrug efficacy are the immunological interactions, the biological barriers that hinder the availability of nanodrugs to the intended target, and the heterogeneity of the biological target (38, 136).

In order to improve their efficacy, nanodrugs should overcome these major limitations and several means of overcoming them are further described in this subsection. An overview of the main issues discussed in this chapter is presented in Figure 1.

**NANODRUGS’ ACTION IS LIMITED BY THE INTERACTION WITH THE BIOLOGICAL SYSTEM**

There are complex interactions of nanodrugs once introduced in a biological system, because they would interact with cellular and humoral constituents of the immune system. Thus, the transition to routine clinical application of these

![Figure 1 Main limitations of nanodrug efficiency in antitumoral therapy and possibilities to overcome the limitations.](image-url)
nanocompounds would be hampered first by different biological barriers and second by their uncertain fate at the diseased site (136, 137).

In the biological system, nanomaterials interact with all the encountered biomolecules and dynamically form the so-called “bio-corona.” The commonly agreed definition of the bio-corona is the multitude and the variety of biomolecules (e.g., proteins, peptides, and lipids) that associate with the surface of a nanoparticle when introduced in a biological system. The process of entrapping nanoparticles within complex surface biomolecules bequeaths them with properties that can hinder the actual intended properties of the nanodrug. Undeniably, the bio-corona establishment controls the nanodrug efficacy and further focuses the actions of natural and adaptive immunity (138). It is not surprising that nanomaterials are directly interacting with the immune system. The evolvement of human immune system was accomplished through exposure to different chemical, physical, and biological agents (139). As NPs are in the size range of biological aggressors, interactions with the immune system are more likely to occur. Thus, a major limitation in nanomedicine is the correct evaluation of the fate of the nanodrugs as antitumoral effectors within a biological system (140–146). As nanomaterials match the same size range as biomolecules and cellular structures endows them with the propensity to reach intracellular structures previously accessible only to biological aggressors. Alternatively, as they have already encountered the complex biological milieu and interacted with other biomolecules, they are subjected to intracellular pathways that are not the intended targets. Hence in vitro investigation of the bio-corona dynamics should be performed to be assured that within the biological system the nanodrugs will reach their intended cellular target (129).

Taking advantage of phagocytes’ capacity to engulf NPs, recently, a novel drug delivery system was reported using macrophages as both carriers and effector cells upon melanoma cells. Hence, nanoparticles of biodegradable photoluminescent poly (lactic acid) were loaded with a drug specific for anti-BRAF V600E mutant melanoma forming a complex engulfed further by macrophages which would directly bind and kill melanoma tumor cells (147–150).

**Conclusion**

Novel treatment methods should have several properties. For example, they should be more effective, cheaper, and without any risk to patient life, even if they do not improve patient’s quality of life. To accomplish patient safety, and for good patient compliance, an ideal treatment should be developed with an improved overall treatment efficiency, a very low possible toxicity, and a specific targeted site (91). Nanotechnology-based formulations can provide all of the above, and their efficacy can be further improved when ornamented with targeting moieties, for instance, specific antibodies (92) or targeted delivery payload (93–95). In the last 5 years, there has been an exponential increase in the focus on nanotechnology with regard to melanoma therapy and related diagnosis (96). Nanomedicine is a new area that develops nanotechnology for therapeutic and diagnostic purposes. Nowadays, different groups of nanomaterials have been designed for drug delivery and/or for identifying specific markers.
Nanomaterials as delivery vehicles can be nanocapsules, nanorods, nanotubes, nanoshells, and nanocages—structures that protect the drug against degradation, thereby enhancing its stability. The development and characterization of PNs, polymeric micelles, liposomes, nanohydrogel, dendrimers, inorganic nanoparticles, and hybrid nanocarriers are among the delivery vehicles that transport different anticancer agents. Chemical drugs, nucleic acids, proteins, antibodies, and functionalization of nanocarriers with inorganic compounds such as magnetic, graphene oxide, carbon, silica, gold and QDs in a core-shell system can generate smart nanodrugs for application in cancer therapy and/or diagnosis. In therapy for skin melanoma, as well as for other tumors, nanotherapeutic strategies deal with several shortcomings that comprise of tumor characteristics, biological barriers, and biocompatibility. Toxicological profile of nanoparticles should be robustly assessed. When systemically administered, nanostructures interact with various host biomolecules, and may trigger toxicity (151, 152). Therefore, comprehensive in vitro cellular models call for evaluation of physicochemical properties, dose, and time of action of nanomaterials, while in vivo assessments would provide valuable data regarding level of absorption, tissue/organ distribution, and metabolism (153). Although preclinical investigations are essential for assessing the potential health risks of nanostructures, animal models retain significant limitations and the human system may react differently to a certain drug compared to animal models (154). Last but not least, the translation of nanodrugs from preclinical to clinical stage is a major issue still unsettled in melanoma nanomedicine area. The future perspectives in nanotechnology applied to cancer is very promising in improving cancer management.

Acknowledgment: Authors Monica Neagu and Carolina Constantin were partially supported through grants PN-II-PCCA-2013-4–1407 (acronym MELTAG, grant no. 190/2014) and COST Action 16120/2017 EPITRAN. Adriana Fretias Neves and Thaise Araujo would like to thank the National Institute of Science and Technology in Theranostics and Nanobiotechnology.

Conflict of interest: The authors declare no potential conflicts of interest with respect to research, authorship, and/or publication of this article.

Copyright and permission statement: To the best of my/our knowledge, the materials included in this chapter do not violate copyright laws. All original sources have been appropriately acknowledged and/or referenced. Where relevant, appropriate permissions have been obtained from the original copyright holder(s).

References


107. Rahimpour Y, Hamishehkar H. Niosomes as carrier in dermal drug delivery: INTECH Open Access Publisher. Tabriz University of Medical Science, Iran; 2012.


