Current and Future Nanotechnology Applications in the Management of Melanoma: A Review

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Abstract

Melanoma is a common malignancy with a high survival rate amongst those diagnosed early. The management of advanced disease is challenging, and current chemosurgery techniques have minimal effect on survival. Nanotechnology, offers great potential in revolutionizing the management of melanoma. New molecules and nanoparticles are designed worldwide in an aim to improve the diagnosis and spread to sentinel lymph nodes and other organs. Novel drug delivery systems are formulated to optimise the distribution and pharmacokinetics of chemotherapeutic agents while reducing their toxic effects. The scope of such molecules extends to therapeutic applications including photodynamic and photothermic therapy where light is converted to heat to combat neoplastic lesions; immunotherapy where nanoparticles are used as immunomodulators or vaccines against cancer cells; and gene therapy which targets pro-oncogenes on signal transduction pathways. This review paper presents current knowledge of the use of nanotechnology in the management of cancer, with a focus on melanoma.

Keywords: Melanoma; Nanotechnology; Quantum dots; C-dots; Theranotics; Photodynamic therapy; Immunomodulators; Malignancies; Epidermis

Introduction

Nanotechnology is a rapidly developing field involving the interdisciplinary study of materials that, at an atomic level, have a size of less than 100 nm. Advances in nanotechnology offer an unprecedented ability to study and manipulate molecular interactions at a sub-cellular level leading to the development of new strategies to image but also treat human disease including cancer.

Skin cancer is the commonest group of malignancies diagnosed in humans. Of this group, malignant melanoma, originating from melanocytes in the basal layer of the epidermis, is the most aggressive form, ranking 6th amongst all diagnosed malignancies [1]. During the last decades, malignant melanoma has become a significant global health problem, particularly amongst Caucasian populations, in which the incidence rate has risen inexorably, nearly quadrupling over the last 20 years [2].

Traditionally, the management of malignant melanoma is considered challenging: its diagnosis is difficult and often patients present late. If managed early, cure rates as high as 97%-99.8% after chemosurgery have been reported [3]. Advanced stages of melanoma are characterized by widespread metastases and currently stage IV disease reports survival rates of less than 10% in 5 years [4]. Conventional treatment options for advanced stages such as surgery, chemotherapy and immunotherapy, although effective in cases of non-metastatic melanomas, are of limited value to disseminated disease [5].

The staging of melanoma is standardized according to the American Joint Committee on Cancer [AJCC] system. Tumour thickness, reported as Breslow Thickness (BT), plays a major role in staging in addition to the ulceration and mitotic rate, the number of nodes involved and the extent of the distant metastasis [6]. Although early stage melanomas (IA) require only a wide local excision, larger or poorly differentiated tumours which are staged as IB and above are offered a Sentinel Lymph Node Biopsy (SLNB), as per the British Association of Dermatology (BAD) guidelines [6].

SLNB is a purely diagnostic test that requires the injection of a radioactive agent and a blue dye to isolate the sentinel lymph node that drains the skin affected by melanoma. An invasive test, SLNB requires the excision of the sentinel node which is consequently biopsied in an attempt to identify melanoma deposits. Although a test of no proven therapeutic value, SLNB comes with inevitable documented surgical risks, the possibility of failure of isolating a sentinel node and a false-negative result.

The MSLT1 study, an international randomized trial on the effectiveness of SLNB on melanoma, has highlighted that although 79.2% of patients did not have nodal disease; those with a positive result (20.8%) had improved survival rates [7]. It is therefore not a coincidence that nanotechnologists have focused on identifying new approaches to SLNB, which are less invasive; carry less surgical morbidity and higher specificity.

More advanced cases require further invasive diagnostic tests. For Stage IIIB and above, CT scans of head, chest, abdomen and pelvis are recommended while Stage IV requires further tests including LDH levels and PET scans [6]. Although not as invasive as SLNB, CT scans carry the inherent exposure to substantially high levels of radiation. Furthermore surgical resection for such cases, (lymph node clearance and/or excision of other melanoma deposits), can be particularly extensive with debilitating consequences for the patient and significant morbidity. The field of photodynamic therapy and laser ablation in association to newly developed nanoparticles might have a role in the future surgical management of melanoma reducing surgical complications and sparing normal native tissue.

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It is becoming increasingly clear that melanoma, like many other cancers, arises due to genetic aberrations that alter signaling pathways that control mitosis, meiosis and apoptosis [8]. Novel therapeutic approaches based on our increasing understanding of the molecular changes that underlie the development of melanoma have recently positively changed the treatment landscape of this disease. Mutation–driven drug development is an ever-optimistic field for the pharmacological treatment of melanoma and has led to the introduction of therapies including Ipilimumab, the BRAF inhibitor Dabrafenib and the MEK inhibitor Trametinib [5].

Chemotherapy itself comes with dire systemic complications that are often so extensive that make the management of advanced cancer futile. Nanotechnology hopes to improve adjuvant oncological approaches to melanoma by creating drug delivery systems that are safe and effective, but at the same time targeted to cancer cells, sparing normal, benign cells.

Immunomodulation is another key advancement in the treatment of malignant melanoma. Immunotherapy involves the blockage of certain monoclonal antibodies expressed solely by the tumour, inhibiting immune tolerance towards the melanoma and possibly leading to its regression. The commonest reported immunomodulators in melanoma are interleukin (IL)-2 and interferon (IFN)-alpha [9-12]. Ipilimumab is one such antibody that is currently in use in Europe and the USA. Such antibodies can also be used as cell targeting systems to further reduce systemic chemotherapeutic effects.

Nanotechnology has the potential to lead to great advances in the arena of melanoma research, increasing the diagnostic and treatment armamentarium, further revolutionizing the clinical approach to melanoma. The epitome of nanotechnology is the evolution of a new field known as theranotics: the design of a single particle which can be used to diagnose stage but also treat cancers such as melanoma. The aim of this paper is to provide an update of current and future uses of nanotechnology in the management of cutaneous melanoma.

Methods

A literature search was performed through Medline, EMBASE, Cochrane Database and Google Scholar for any previous research applications pertaining to applications of Nanotechnology to the management of Malignant Melanoma until August 2015. The terms used were melanoma, nanotechnology and nanoparticles for prospective and retrospective studies in the English Language to include animal studies. The search was limited to articles presenting or evaluating nanoparticles being investigated as both diagnostic but also treatment options for melanoma. Exclusion criteria were case reports, reviews and articles investigating nanotechnology use for other malignancies. The references of the included studies were screened to identify potential citations not captured by the MEDLINE search. Validation of search results was conducted by two separate reviewers (A.S and H.L.H.). Any discrepancy was reconciled by a third reviewer (A.P.). After the group of abstracts was agreed upon, these articles were reviewed in their entirety and selection of articles to include in this systematic review was based on each article meeting the criteria previously listed. An additional search of Trials underway globally was performed on www.clinicaltrials.gov and the results were incorporated in our study.

Results

Nanotechnology in diagnostics

Early diagnosis of malignant melanoma is key resulting in wide local excision and high survival rates [6]. The aim of using these nanoparticles is to achieve high specificity and sensitivity in the various diagnostic tests used, whilst reducing invasiveness and morbidity.

Quantum Dots [QD]: "Quantum Dots", are fluorescent nanoparticles composed of semiconductor nanocrystals that are being trialed in the detection of melanoma [13,14]. QDs are semiconductor crystals with optical and electrical properties that are relative to the energy released from excitons transitioning between energy levels. Their narrow field of emission and broad absorption spectra offer great photostability: at the near infrared region they emit at wavelengths ideal for deep tissue imaging thus making them useful in detection of cutaneous melanoma [15].

To improve targeting of cancer cells, various groups have studied the potential of Quantum Dots [QD] with molecules or antibodies specific to certain cancers in an aim to enhance their diagnostic properties and increase specificity. Already QDs have been conjugated with anti-HER2 monoclonal antibodies and have shown promising results in detecting breast tumours and promoting image guided surgery [15].

A research group lead by Zheng was able to detect melanoma *in vitro* by coupling Cadmium and Zinc [CdSe/ZnS] QD with CD146, an antigen that is over-expressed in melanoma cells [16]. The tumour cells were distinctly imaged separate from native melanocytes in a vitro setting.

Antibody-conjugated QD have been investigated as a means of imaging cells and labeling those undergoing apoptosis, separate from cells that are not. Antibodies generated against a human apoptotic peptide, calreticulin, conjugated to a specially coated QD has shown enormous potential in cancer detection and visualization [17]. *In vivo* use of QD has been practiced in mice to accurately localize human prostate cancer and hepatocellular cancer xenografts [18].

Despite their great optical and imaging potential, to date no animal studies or early phase clinical trials are published with QD used as an imaging modality for cancer. This is likely due to the toxicity, thrombogenicity and immunogenicity related to the heavy metal composition of QD [19-21].

In an attempt to overcome toxicity, many units have designed biologically compatible surface coating devices which unfortunately also raise concerns related to excretion and systemic sequestration. A novel device makes use of a polyhedral oligomeric silsesquioxane [POSS]-coated CdTe-cored QD using mercaptosuccinic acid [MSA] and D-cysteine as stabilizing agents [22]. The amphiphilic nature of this device renders it soluble in aqueous solutions and cell membranes thus enabling the use of lower QD concentrations to avoid their toxic sequelae [22].

Another limitation of QDs is their ability to penetrate cell membranes. New research in nanotechnology aims in bypassing this limitation by conjugating QD to Tat, a cell penetrating peptide sequence derived from human immunodeficiency virus [HIV]-1 [23]. As these Tat-conjugates are taken up by endocytosis and often entrapped with endosome lysosomes, extensive research, including the use of phthalocyanine photosensitisers is underway to improve bioavailability [23].

Cornell dots: Cornell Dots, another form of nanoparticles better known as C-Dots, have been used as probes in sentinel lymph node biopsy [24]. They are silica-based nanoparticles with a polyethylene

glycol [PEG] shell which neutralizes their charge, preventing them from being scavenged by the liver, spleen and bone marrow. Their targeting peptide is the RGD that attaches to alpha 2 beta 3 integrin which is overexpressed in melanoma cells [25]. They are FDA approved and a group lead by Bradbury has made significant progress in the field of nanomedicine and melanoma, demonstrating in murine models that when these particles were injected in the presence of PET and optical imaging they were successful in detecting and localizing melanoma positive sentinel lymph nodes [24]. Theoretically C-Dots may serve a role in determining the tumour burden of positive nodes, as well as a molecular marker that can be used to monitor the response of ablative treatment.

As an exciting development C-dots are now in the early phases of clinical trials in the USA [26]. In the first human trial, technetium-99m (99mTc) sulphur colloid administered preoperatively and intraoperatively injected fluorescent cRGDY-PEG-Cy5.5-C dots are used for the identification of lymphatic spread to sentinel nodes. Intraoperative Near Infra Red (NIR) fluorescence imaging is performed using a hand-held fluorescence camera system, along with gamma counting for 99mTc sulfur colloid. Results are then histologically confirmed in resected tissue specimens [26].

Gold nanoparticles: Another important nanoparticle in this field is gold; particularly with it being considered toxically benign. Currently, melanoma targeted gold nanocages have been used in photoacoustic tomography with high specificity due to an enhancement in imaging resolution [27]. Gold nanoparticles directly labeled with the radioisotope indium-111 and conjugated again with RGD-based ligands have been used successfully in mice as radiotracers of melanoma [27]. Indium -111 is already approved for use in non-Hodgkins lymphoma [28]. These nanoparticles show great clinical promise in cancer imaging due to their high stability as labels, but also due to their low interference with the biological profile of melanoma [29,30] Unfortunately with regards to using gold to enhance CT imaging, the concentration of the gold nanoparticles required to deflect unwanted portions of the x-ray is currently impractically high [31].

Magnetic nanoparticles: In contrast to gold nanoparticles, magnetic nanoparticles [MNPs] have recently had great success in cancer diagnostics in clinical trials when used in conjunction with MRI [31]. In view of the positive early results of these trials, the use of MNPs has been extended to melanoma.

The MELAMAG trial forms a prospective multicenter feasibility non-randomised clinical trial comparing sentinel node biopsy using magnetic nanoparticles versus the standard technique. The basis of the new technique is to use an intradermal injection of magnetic nanoparticles [Sienna+] and a hand held magnetometer [SentiMag] to detect sentinel node[s] intraoperatively. Should this trial prove MNPs to be as effective as the standard technique, it has the potential to revolutionize imaging in melanoma by eliminating the use of radioactive materials and their associated hazards.

Superparamagnetic Iron Oxide [SPMIO]: This molecule travels via lymphatics to lymph nodes and acts as a negative contrast for T2 weighted MRI sequences with the metastatic deposits highlighted as white [32,33]. A study of the use of this particle in SLNB in breast cancer showed that SPMIO-MRI has the potential for an in *vivo* preoperative detection of metastatic lesions [32,33]. Similar success has been demonstrated in the use of super-paramagnetic carbodextran-coated iron oxide nanoparticles [34]. A prospective multicentre clinical trial comparing standard sentinel node biopsy using these magnetic nanoparticles versus standard use of patent blue dye and radioisotope in patients with breast cancer requiring SLNB is underway in the United Kingdom.

Nanotechnology and melanoma treatment

Drug delivery: Chemotherapy makes use of cytotoxic drugs to destroy highly proliferative cancer cells as well as normal cells that are in their division phase [35]. To date there is no evidence to show a survival benefit when using adjuvant chemotherapy in patients with metastatic melanoma and is consequently only used in the higher stages of disease [36-38].

The major disadvantage of all chemotherapeutic agents is cytotoxicity - they also kill normal cells leading to the well known side effects of reduced immunity, gastrointestinal inflammation and hair loss; these combined with low response rates are far from ideal [35]. Drug delivery methods can be used not only to reduce systemic side effects but to also improve pharmacokinetics in an attempt to make chemotherapy more user-friendly.

A study performed on an animal model using doxorubicin delivered via a fullerene nanoparticle shows similar efficacy to that of the free agent, but free from the undesirable toxic effects [39]. Additionally, this nanoparticle helped maintain a sustained drug release to the affected tumour [40]. Drugs encapsulated in delivery systems are able to remain in the blood stream longer, enabling sustained drug release, also providing more accurate tumour targeting [41,42].

In order to be effective drug delivery systems, nanoparticles are designed with multiple properties: high affinity to therapeutic agents, a stability within human serum, a propensity to be attracted to and bind on cancer cells specifically via ligands [43] to allow offloading of the named drug and safe degradation [44]. Some have the added advantage of delivering more than one drug at one time, hence allowing for combination therapy [45].

Drug delivery systems are classified as either active or passive targets [43]. Active systems involve specific interactions between the drug-loaded nanoparticles and tumours. This requires targeting nanoparticles with ligands specific to tumour cells. Such ligands include monoclonal antibodies, peptides, and nucleic acids [43]. Passive drug delivery systems rely on the permeation and retention effect whereby the leaky vessels and poor lymphatic drainage in tumours allows for increase drug accumulation [43]. To date, there are only four FDA approved nanoparticle-based cancer drug delivery systems but none of these is licensed for melanoma. Promising research is however under way.

Liposomes: Liposome based drug conjugates form a striking example of an effective nanoparticles based drug delivery system. They are artificial vesicles consisting of an aqueous core and outer phospholipid layer which can act as vessels for various drugs. Currently in the US and UK, liposome coated Doxorubicin [Doxil US, Myocet UK] is in use for ovarian cancer and myeloma. Unfortunately phase II trials of Doxil in melanoma therapy were discontinued due to ineffectiveness [46]. *In vitro* studies have been performed with docetaxel conjugated with carboxymethyl chitosan showing improved antitumour effect in B16 bearing melanoma mice [43]. Similarly a group led by Zhang et al. combined paclitaxel with polymeric micelles and found significant prevention in tumour growth, and increased survival time in subcutaneous and pulmonary B16-F10 melanoma mice [43].

PEG micelles: The most promising development for melanoma and liposomes is the Phase I trial underway in Japan involving NC-6004, Cisplatin incorporating micelles. Cisplatin has previously been used in melanoma but is often withheld due to its neurotoxic and nephrotoxic side effects [46]. NC-6004 consists of a PEG, a complex of miscelles [similar to liposomes] and the drug Cisplatin. Animal work has been effective in demonstrating that in this form Cisplatin has greater accumulation in solid tumours but much less so in its distribution in normal tissue [47].

Albumin micelles: Polymeric nanoparticles particularly those made of albumin have shown promise as drug delivery systems for melanoma. Paclitaxel bound to an albumin derived nanoparticle, Abrexane, has been approved by the FDA since 2005 for the treatment of metastatic breast cancer [48]. Eight clinical trials have also been approved to investigate albumin bound paclitaxel for metastatic melanoma either alone or in conjunction with other drugs. To date the results of early phase clinical trials for chemotherapy naïve patients are promising.

Antibody-drug conjugates: Another nano-technique involved in drug delivery makes use of Antibody-Drug Conjugates (ADCs). ADCs are drug molecules conjugated to a single antibody used to target a molecule overexpressed on cancer cells. Usually these antibodies are conjugated with a drug that induces cell death. While this concept appears straightforward, only 2 FDA approved ADCs are available (Brentuximab vedotin and Trasruzumab emtansine) but none of these two trials are investigating their use in melanoma [49].

Studies have made use of this concept on murine models. Falvo et al. synthesized a novel antibody-drug conjugate incorporating ferritin-based nanoparticles. Monoclonal antibody (mAb) Ep1 to the human melanoma-specific antigen CSPG4 were conjugated successfully to a single ferritin cage encapsulating cisplatin molecules (HFt-Pt-Ep1) causing tumour cell death [49].

Various nanoparticles loaded with different combinations of drugs targeting melanoma are continuously being developed indicating great hope for the future of nano-drug delivery platforms for advanced melanoma. These are summarized in Table 1.

Molecular targeting, gene silencing and cell targeting: Emerging evidence links the development of melanoma and its metastasis to mutations in genes encoding CDK4, MCIR, and CDKAN2A, somatic mutations in pro-oncogenes B-RAF, N-RAS and KIT as well as tumour suppressor genes PTEN, P53 and CDKN2A. Recent advances in understanding these key mutations have opened the door to the development of new therapeutic treatments. These technologies either target melanoma specific mutations and pathways, or cell surface markers on the tumour cells in an attempt to improve drug delivery, allowing for gene silencing or the design of vaccines to target tumours.

Nanoparticles and gene silencing: Gene silencing therapy involves the use of small interfering RNA (siRNA), plasmid DNA or antisense oligonucleotides to target certain molecular chains inhibiting gene expression [50,51]. Of these techniques, siRNA's have shown most promise. siRNAS are double stranded RNA molecules that have the capacity to interfere with the translation of specific mRNAs based on their sequence. They interact with an Argonaute (a catalytic portion of an RNA-induced silencing complex) which unwinds the molecule and also degrades its sense strands to prevent the process of translation. The antisense strand then binds onto the complementary mRNA of the desired gene sequence targeting it to further be degraded by the Argonaute. This process of gene silencing makes use of a process known as RNA interference [51].

As with the previously described drug therapies, siRNAs are not without their limitations. The most significant problem is an inability to deliver sufficient siRNA into the cytoplasm of target cells, while other issues include accidental silencing of non-intended, non-target mRNA and inflammation [52].

Nanoparticles have been developed which are shown to carry and protect the siRNA, specifically delivering it to their target cells and together with their contained target, the siRNA can be efficiently taken up into the cell. Nanolipids and gold nanoparticles are thought to be the most promising molecules for clinical application [53].

Undoubtedly, the most researched signaling cascade in melanoma is the MAPK-ERK pathway (mitogen-activated protein kinase-extracellular-related kinase) [54]. siRNA inhibitors have been trialed in an aim to silence mutations in this pathway that are linked to the development of melanoma. siRNA lysosomal nanoparticles have already been used to effectively silence the STAT3 gene (signal transducer and activator of transcription 3) in B16 melanoma cells; reducing melanoma cell viability to 16% after only 3 doses [55].

The first human clinical trial in this field involved CALAA-01, an siRNA molecule that makes use of a cyclodextrin- polymer based nanoparticle. This trial demonstrated that siRNA administered systemically to humans can produce a specific gene inhibition by means of RNA interference [56].

Anti-melanoma vaccines and cell targeting: Targeting of melanoma cells with nanoparticles is another desirable treatment modality. Melanoma specific cell surface markers have been identified and expressed to allow successful nanoparticle -targeting. In theory, such target molecules must be expressed at very high levels on melanoma cells but at very low or ideally negligible levels on normal cells. This would allow specific drug delivery systems to operate but also anti-melanoma vaccines making use of gene silencing techniques.

Phase 1 trials using siRNA and nanoparticles confirmed the safety and efficacy of an anti-melanoma vaccine [57]. An siRNA- nanoparticle combination has been used against BRAF and Akt3 effectively inhibiting cutaneous melanocytic lesion development [58]. Similarly an antisense oligonucleotide against an anti-apoptotic protein Bcl-2, Oblimersen, showed a decrease in melanoma growth *in vitro* and in *vivo* [59].

Used as a combination therapy in a Phase 1 trial with 2 other albumin coated chemotherapeutic agents, this gene silencing vaccine has proven to more effective against advanced stage melanoma [60]. Basu et al. showed that nanoparticle-mediated targeting of MAPK signaling inhibits proliferation and induces apoptosis of melanoma cells, also enhancing the effect of chemotherapy on these cells [61].

MC1R [melanocortin-1 receptor] which is expressed on melanocytes but at a much higher concentration on melanoma cells is one such ligands that nanoparticles can use to permeate cell targeting [62]. Lu et al. have used a murine model to successfully conjugate gold nanospheres to the alpha-melanocyte-stimulating hormone (MSH) analog, a potent agonist of MC1R. The targeted melanoma cells can then be eliminated via photothermal ablation, a concept which will be discussed further in this review [63].

Immunotherapy: Cancer immunotherapy aims at improving the immune system of a patient in order to increase the clearance of cancer cells from the body. This can be achieved via cell-mediated,

NANO-technology	Study ID	Study Title	Phase	Drug	Study design	Study arms	Condition
Immunotherapy	NCT02054520	Immunotherapy Study for Patients With Stage IV Melanoma	IIB	HyperAcute®-Melanoma (HAM) Immunotherapy	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	Experimental: HyperAcute®-Melanoma (HAM) Immunotherapy + Ipilimumab Active Comparator: Ipilimumab Alone	Stage 4 melanoma
Polymeric Nanoparticles	NCT02158520	Paclitaxel Albumin-Stabilized Nanoparticle Formulation and Bevacizumab or Ipilimumab as First-Line Therapy in Treating Patients With Stage IV Melanoma That Cannot Be Removed By Surgery	II	paclitaxel albumin-stabilized nanoparticle formulation	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Crossover Assignment Masking: Open Label Primary Purpose: Treatment	Experimental: Arm A (nab-paclitaxel and bevacizumab) Experimental: Arm B (ipilimumab)	• Stage IIIA Melanoma • Stage IIIB Melanoma • Stage IIIC Melanoma Stage IV Melanoma
Polymeric Nanoparticles	NCT00626405	Bevacizumab and Temozolomide or Bevacizumab and Paclitaxel Albumin-Stabilized Nanoparticle Formulation and Carboplatin in Treating Patients With Stage IV Malignant Melanoma That Cannot Be Removed by Surgery	II	Biological: bevacizumab Drug: carboplatin Drug: paclitaxel albumin-stabilized nanoparticle formulation Drug: temozolomide	Allocation: Randomized Primary Purpose: Treatmen	Experimental: Arm I Interventions: • Biological: bevacizumab Drug: temozolomide Experimental: Arm II Interventions: • Biological: bevacizumab • Drug: carboplatin Drug: paclitaxel albumin-stabilized nanoparticle formulation	MELANOMA
siRNA	NCT00689065	A Phase I, Dose-Escalating Study of the Safety of Intravenous CALAA-01 in Adults With Solid Tumors Refractory to Standard-of-Care Therapies	I	CALAA-01: a small interfering RNA (siRNA). This siRNA inhibits tumor growth via RNA interference to reduce expression of the M2 subunit of ribonucleotide reductase (R2). The CALAA-01 siRNA is protected from nuclease degradation within a stabilized nanoparticle targeted to tumor cells.	Endpoint Classification: Safety Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatmen	Experimental: CALAA-01 Intervention: Drug: CALAA-01	Solid Tumours
Polymeric Nanoparticles	NCT00404235	Carboplatin and ABI-007 in Treating Patients With Stage IV Melanoma That Cannot Be Removed By Surgery	ii	• Drug: carboplatin • Drug: paclitaxel albumin-stabilized nanoparticle formulation	Masking: Open Label Primary Purpose: Treatment	This phase II trial is studying the side effects and how well giving carboplatin together with ABI-007 works in treating patients with stage IV melanoma that cannot be removed by surgery.	Melanoma
Quantum Dots	NCT02106598	Targeted Silica Nanoparticles for Image-Guided Intraoperative Sentinel Lymph Node Mapping in Head and Neck Melanoma, Prostate and Cervical/Uterine Cancer Patients	0	fluorescent cRGDY-PEG-Cy5.5-C dots	Endpoint Classification: Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Diagnostic	Melanoma patients will be injected with a radioactive dye around the tumor site, and images will be acquired about 2 hours the location of the later using a device to image the dye.	

ADC (antibody drug conjugates)	NCT02129075	CDX-1401 and Poly-ICLC Vaccine Therapy With or Without CDX-301in Treating Patients With Stage IIB-IV Melanoma	ii		Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Parallel Assignment Masking: Open Label Primary Purpose: Treatment	This randomized phase II trial studies how well DEC-205/NY-ESO-1 fusion protein CDX-1401 (CDX-1401) and neoantigen-based melanoma-poly-ICLC vaccine (poly-ICLC) vaccine therapy work when given with or without recombinant flt3 ligand (CDX-301) in treating patients with stage IIB-IV melanoma	
Immunotherapy	NCT01814046	Adoptive T Cell Therapy for Metastatic Ocular Melanoma	II	Drug: Aldesleukin	Allocation: Non-Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	Participants will receive an infusion of their collected white blood cells. They will also receive aldesleukin for up to 5 days to boost the immune system's response to the white blood cells.	• Metastatic Ocular Melanoma Metastatic Uveal Melanoma
Immunotherapy	NCT01863108	Safety Study of a Dendritic Cell-based Cancer Vaccine in Melanoma	i	GeniusVac-Mel4:a drug product composed of an irradiated allogeneic plasmacytoid dendritic cell (PDC) line loaded with 4 melanoma peptides derived from Melan-A, gp100, Tyrosinase or Mage-A3	Endpoint Classification: Safety Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	Experimental: GeniusVac-Mel4 Sub-cutaneous injections of GeniusVac-Mel4 in patients with melanoma. Intervention: Biological: GeniusVac-Mel4	melanoma
Quantum Dots	NCT01266096	PET Imaging of Patients With Melanoma and Malignant Brain Tumors Using an 124I-labeled cRGDY Silica Nanomolecular Particle Tracer: A Microdosing Study		PET scan with 124I-cRGDY-PEG-dots	Allocation: Non-Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Diagnostic		• Newly Diagnosed or Recurrent Metastatic Melanoma Patients Malignant Brain Tumors
siRNA	NCT01941927	Trametinib With GSK2141795 in BRAF Wild-type Melanoma	Ii	• Drug: Trametinib (GSK1120212) Drug: GSK2141795	Endpoint Classification: Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment		
Tumour targetting	NCT02236546	FDG-PET/CT as a Biomarker for Treatment Response in Advanced Melanoma		18F] fluorodeoxyglucose (FDG)	Endpoint Classification: Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Diagnostic	Patients undergo [18F] fluorodeoxyglucose (FDG) positron emission tomography (PET)/computed tomography (CT) up to 2 weeks prior to first dose of therapy, after completion of the first treatment course (day 21), and after completion of the fourth treatment course (day 84).	• Recurrent Melanoma • Stage IIIA Melanoma • Stage IIIB Melanoma • Stage IIIC Melanoma Stage IV Melanoma
Imaging	NCT02106598	Targeted Silica Nanoparticles for Image-Guided Intraoperative Sentinel Lymph Node Mapping in Head and Neck Melanoma, Prostate and Cervical/Uterine Cancer Patients	0	fluorescent cRGDY-PEG-Cy5.5-C dots	Endpoint Classification: Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Diagnostic	Melanoma patients will be injected with a radioactive dye around the tumor site, and images will be acquired about 2 hours the location of the later using a device to image the dye.	• Head and Neck Cancer • Melanoma • Prostate Cancer • Cervical Cancer Uterine Cancer

Imaging		Sentinel Node Biopsy using Magnetic Nanoparticles for melanoma		Sienna+	Detection rate with either the standard (blue dye and isotope) or the new technique (magnetic) Timepoint(s): The proportion of sentinel nodes detected (detection rate) with either the standard or the new magnetic technique	Non-randomised; Interventional; Design type: Diagnosis	melanoma
siRNA	NCT00672542	Immunotherapy of Melanoma With Tumor Antigen RNA and Small Inhibitory RNA Transfected Autologous Dendritic Cells	I	Proteasome siRNA and tumor antigen RNA-transfected dendritic cells	Allocation: Non-Randomized Endpoint Classification: Safety Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	• Experimental: A siRNA loaded dendritic cells derived from untreated monocytes Intervention: Biological: Proteasome siRNA and tumor antigen RNA-transfected dendritic cells • Experimental: B Vaccination with melanoma tumor associated antigen RNA loaded dendritic cells derived from monocytes transfected with control siRNA Intervention: Biological: Proteasome siRNA and tumor antigen RNA-transfected dendritic cells Experimental: C Vaccination with melanoma tumor associated antigen RNA loaded dendritic cells derived from monocytes transfected with siRNA targeting the three inducible immunoproteasome subunits Intervention: Biological: Proteasome siRNA and tumor antigen RNA-transfected dendritic cells	Metastatic Melanoma with Absence of CNS Metastases
siRNA	NCT02166255	APN401 in Treating Patients With Melanoma, Kidney Cancer, Pancreatic Cancer, or Other Solid Tumors That Are Metastatic or Cannot Be Removed By Surgery	I	siRNA-transfected peripheral blood mononuclear cells APN401	Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	Experimental: Treatment (APN401)	• Recurrent Melanoma • Recurrent Pancreatic Cancer • Recurrent Renal Cell Cancer • Stage III Pancreatic Cancer • Stage III Renal Cell Cancer • Stage IIIA Melanoma • Stage IIIB Melanoma • Stage IIIC Melanoma • Stage IV Melanoma • Stage IV Pancreatic Cancer • Stage IV Renal Cell Cancer Unspecified Adult Solid Tumor, Protocol Specific

Table 1: Current clinical trials making use of nano-technology in melanoma (as per www.clinicaltrials.gov) (101).

antibody and cytokine therapies. These agents target cancer antigens – molecules similar to cell surface markers that cancer cells specifically express; nominally proteins but also other molecules such as carbohydrates. Immunotherapy aids the body in clearing cells damaged by chemotherapy and targeted therapy to improve clearance and resolution [64,65].

Antibody therapy: Antibody therapy is the most successful form of immunotherapy, but currently there is no cell-based therapy approved for melanoma. The phase 3 trials previously performed have had disappointing results but research is still ongoing and 19 trials are registered to date. Current research is focused on Ipilimumab a monoclonal antibody that blocks CTLA-4 found on the surface of T-cells. Designing a nanoparticle that carries and delivers this monoclonal antibody to T –cells may be the next stage in improving cytotoxic responses, decreasing side effects, inflammation and even further prolonging survival from advanced melanoma.

Stephan et al. [66] have designed maleimide-functionalized nanoparticles that are able to bind to free thiol groups on T-cell membrane proteins. This is anefficient way of delivering compounds onto the T cell synapse, and promoting T –cell expansion at the tumour site. Another study conducted in Japan found that N-propionyl cysteaminyl phenol-maleimide-dextran [NPCMD] stimulated CD14$^+$monocytes and THP-1 cells to secrete TNFα, IL-6 and IL-8, but not IL-10 or IL-12. The immunopotentiating effect of NPCMD mediated by TLR4 and NLRP3 inflammasome activation could be used as method of potentiating an effective adaptive immune response against melanoma [66].

Another form of immunotherapy is mediated via the action of IL2 and INFα, which are immunomodulators that stimulate the immune system to attack abnormal cells found in cancers like melanoma. Such form of immunotherapy, however, has a wide range of side effects. Yao et al. designed a polyethylenimine based nanoparticles which was linked to folate conjugated beta-cycodextrin and interleukin-2 plasmid, in an aim to reduce the toxic effects of these immunomodulators. Murine studies of this agent show a regression of melanoma growth and increased survival [67].

Similarly, Cejudo-Guillen et al. [68] successfully used a nanoporous miniature device for local delivery of INFα achieving a constant slow release and hence avoiding severe side effects.

Photodynamic therapy: Photodynamic therapy (PDT) has recently emerged as another therapeutic option in treating cancer. It makes use of cytotoxic oxygen-based molecules in order to promote cell death. It uses a photosensitizer, a chemical activated by light of a certain wavelength, to generate cytotoxic oxygen based molecular species such as oxides (O_2), superoxides (O_2^-) and peroxides (OH) [69]. These reactive molecules damage subcellular organelles and plasma membranes of cells resulting in their death.

The role of nanotechnology is to improve PDT by making it more targeted and efficient, using nanoparticles that are either active or passive. Passive PDT nanoparticles are carriers of photosensitizers only. Active PDT nanoparticles can themselves generate reactive molecules without the need for a photosensitizer [69].

Navarro et al. designed nanocarriers composed of biocompatible polymers grafted on gold nanospheres. When labeled with a fluorescent photosensitizer, they can trigger cell death in melanoma enhancing the activity of passive PDT [70]. Vijayaraghavan et al. also showed that gold nanoshells can absorb near-infrared light, emit fluorescence, form

oxygen free radicals and induce nanomaterial mediated photodynamic therapy [NmPDT] leading to complete destruction of solid melanoma tumors in mice [71]. Samia et al. using semiconductor Quantum dots was the first to introduce the concept of nanoparticles as active generators of singlet oxygen molecules and much work is now evolving in this field although this is yet to be taken to clinical trials phase [72].

Photothermal therapy: Photothermal therapy [PTT] uses photothermal agents to achieve selective and controlled heating of target cancer cells, confining thermal damage to the tumour only. Gold nanoparticle and carbon nanotubes have strong absorption in the near-infra red regions of the electromagnetic spectrum making them ideal for *in vivo* PTT. The use of these nanoparticles has lead to lower laser energy requirements allowing for only local cellular destruction and death.

Primarily, the photosensitizer nanoparticles must accumulate within the target tumour and this can be achieved by functionalizing the nanoparticles with specific tumour targeting molecules. Jung et al. have synthesized a nanographene oxide-hyaluronic acid conjugate (NGO-HA) for photothermal ablation of melanoma [73]. This was delivered transdermally to the tumour in mice skin and was then irradiated with a near-infrared laser (NIR). Laser irradiation resulted in the tumour being ablated completely and no recurrence was seen. In a similar study, Chu et al. injected fluorescent quantum dots – CdTe(710) QDs coated with silica and showed that the growth of murine melanoma tumours was significantly inhibited after laser irradiation, with eventual disappearance of the tumour [74].

The positive results of PTT either alone or in conjunction with other treatments shows great promise for the future. Although no study has been translated into a clinical trial for melanoma it must only be a matter of time, as PTT making use of AuroShell (gold metal shell and silica cover) has entered human trials in the management of neck and lung cancer.

Theranostics

Theranostics describes the ability of substances such as nanoparticles to be used for simultaneous diagnosis and treatment. The ideal theranostic nanoparticle would be one that could diagnose melanoma, deliver a targeted therapy and then assay the response of the melanoma to this therapy. As biochemical techniques have improved dramatically, it is now possible to create nanoparticles that are multidimensional, having both hydrophilic and hydrophobic facets allowing them, for example, to carry a hydrophilic contrast and hydrophobic drug.

Theoretically, the combinations are limitless. Multimodal nanoparticles have been designed to have a superparamagnetic core to allow imaging with MRI and a gold shell to facilitate PTT. [75] Similarly silica –coated gold nanorods have strong attenuation for XRay and CT imaging and can also be used for PTT. SPIONs used in MRI can be loaded with a chemotherapeutic drug or gene delivery carrier and then tracked with MRI. Gold nanoparticles can be combined with photosensitizers for dual modality treatment - PDT and PTT.

Wu et al. developed hybrid nanogels by coating a Ag-Au bimetallic NP core with a thermo-responsive nonlinear poly (ethylene glycol) (PEG)-based hydrogel as shell [76]. The Ag-Au NP core induced fluorescence and was used in mice for imaging and identifying melanoma B16F10 cells. The reversible thermo-responsive volume phase transition of the nonlinear PEG-based gel shell was able to modify the physicochemical environment of the Ag-Au NP core providing a

Nanoparticle	Description	Clinical Use	Side Effects	Studies
Nanoparticles Used in Diagnosis of Melanoma				
Quantum Dots [20,21,22]	Cadmium and Zinc (CdSe/ZnS) QD with CD146, an antigen that is over-expressed in melanoma cells	Targetting of melanoma cells for diagnostic and imaging purposes.	Thrombogenicity and immunogenicity	n/a
Quantum Dots [28]	Polyhedral oligomeric silsesquioxane (POSS)-coated CdTe-cored QD using mercaptosuccinic acid (MSA) and D-cysteine as stabilising agents	The amphiphilic nature of this device renders it soluble in aqueous solutions and cell membranes enabling the use of lower QD concentrations hence avoiding toxicity.	n/a	n/a
C- Dots [30,31]	Silica Based nanoparticles with poly-ethylene glycol (PEG) shell	Enhancement of Sentinel Lymph Node Biopsies (SLNB)	FDA approved	Clinical Trial IND approved Phase 0 clinical trial (since 2011)
C Dots [32]	technetium-99m (99mTc) sulphur colloid and fluorescent cRGDY-PEG-Cy5.5-C dots	Tumour localisation and detection using fluorescence camera.	In trial	Clinical Trial stage Phase O NCT02106598
Gold Nanorods [33]	gold nanorods conjugated with Arg-Gly-Asp peptides (RGD-GNRs)	Enhance the response of melanoma cells to 6 mV radiation.	Toxicity related to ligands on gold nano-particles	*In vitro*
Magnetic Nanoparticles [37]	Sienna+ magnetic nanoparticles	Enhancement of Sentinel Lymph Node Biopsies (SLNB)	Rash Itchiness Dizziness Skin discolouration	MELAMAG study
Superparamagnetic iron oxide (SPIO) [38,39]	SPIO	Enhancement of Sentinel Lymph Node Biopsies (SLNB)	n/a	SENTIMAG study
Superparamagnetic carbodextran-coated iron oxide [40]	DS-SPION	Enhancement of MRI/PET scan	n/a	*In vitro*
Drug Delivery Systems				
Liposomes [52]	Cisplatin	Artificial vesicles consisting of an aqueous core and outer phospholipid layers; their aqueous core can be filled with drugs	Toxicity due to cationic nature;	Doxil (US) Myocet (UK) used for ovarian CA and myeloma
	Vincristine			
	Doxorubicin		Hand-foot syndrome	Clinical trial for melanoma showed ineffective.
				NC-6004 (liposome-coated Cisplatin) phase I trial Japan
Albumin-coated nanoparticles [55]	Paclitaxel with Abrexane (albumin coat)	Drug Delivery System	n/a	8 clinical trials underway for melanoma
Antibody Drug Conjugates (ADCs) [56]	Brentuximab vedotin, Trasruzumab emtansine	Drug Delivery System	n/a	No clinical studies in melanoma
Antibody Drug Conjugates (ADCs) [56]	Monoclonal antibody (mAb)Ep1 bound to ferritin cage encapsulating cisplatin *HFt-Pt-Ep1)	Binds to the human melanoma specific antigen CSPG4	n/a	n/a
Molecular Targeting				
siRNA [61-63,68]	CALAA-01	Interfere with DNA and protein synthesis and work by gene silencing	Silencing on unintended RNA/ unable to transfer enough siRNA	CALAA-01 trial
	GSK2141795			
	APN401			
	Dendritic cells			
Melanoma specific cell marker [69,70]	gold nanospheres to the alpha-melanocyte-stimulating hormone (MSH) analog, a potent agonist of MC1R	Used as cell markers and photothermal ablation.	n/a	n/a
Immunotherapy				
Vaccine [74]	HAM (hyperacute melanoma immunotherapy)	The cellular components (HAM-1, HAM-2, and HAM-3) of HyperAcute-Melanoma immunotherapy have been derived from allogeneic melanoma cancer cell lines.	Asthenia, Chills, dehydration, diarrhoea, fatigue, malaise	HAM trial
Immunomodulator delivery vector [72]	N-propionyl cysteaminylphenol-maleimide-dextran (NPCMD)	The immuno-potentiating effect mediated by TLR4 and NLRP3 .	n/a	n/a

Nanopolymer [75]	low molecular weight polyethylenimine (600 Da) linked by β-cyclodextrin and conjugated with folate (named H1)	Nanopolymer used to deliver IL2 to affected tissues hence stimulating activation and proliferation of CD8+, CD4+ T cell, and natural killer cells and increasing the integration of these into the tumor environment	IL2 – side effects: flu-like symptom hypotension nausea/vomiting diarrhea fatigue confusion shortness of breath rash low urine output	n/a
Drug delivery system	Nanoporous Silica Microparticle (NSiO(2)-MP)	A drug delivery system and scaffolding platoform that activates Toll like Receptors (TLR) and macrophages.	As above	n/a
Vaccine	Exosomes	Exosomes can be used for both diagnostic and therapeutic agents (vaccine)	n/a	n/a
Photodynamic Therapy				
Nanocarriers	Shells of charged biocompatible polymers grafted on gold nanospheres	They can be labelled with a fluorescent photosensitizer and offer high local loading enhancing the use of Photodynamic Therapy	n/a	n/a
Nanomaterial-induced photodynamic therapy [81]	Gold nanoechinus	Gold nanoechinus can form singlet oxygen leading to photodynamic and photothermal therapeutic effect	n/a	n/a
Photothermal Therapy				
Delivery carrier [80]	Nanographene oxide-Hyaluronic Acid conjugate (NGO-HA)	Transdermal delivery of NGO-HA to tumor tissues enabling the enhanced penetration of NIR irradiation.	n/a	n/a
Theranostics				
Nanogels [82]	Ag-Au bimetallic NP core with a thermo-responsive nonlinear poly(ethylene glycol) (PEG)-based hydrogel shell	Imaging; release of temozolimide to target cells; photothermal therapy	n/a	n/a
Carbon Nanotubes [81,82]	a tubular structure composed of sheets of benzene rings	Used as contrast agent for PET scans but also as biomarkers.	n/a	n/a
Magnetic Carbon nanotubes [80-82]	As above	They are conjugated to chemotherapeutic agents to provide treatment of lymphatic melanoma metastasis and SNLB (88). There is also a great scope for use as drug delivery systems, photodynamic therapy and gene	n/a	n/a

Table 2: Various nanoparticles loaded with different combinations of drugs targeting melanoma are continuously being developed indicating great hope for the future of nano-drug delivery platforms for advanced melanoma.

high loading capacity for temozolomide and offer a thermo-triggered drug release. The scope for nanoparticles as theranostics is endless.

Exosomes, small biological membrane vesicles, are molecules of double-edged features that are being investigated as both diagnostic and therapeutic agents due to their potential use as biological markers for certain cancers, as well as drug delivery systems. They have also been studied as agents that could formulate pioneer cancer vaccines that could control carcinogenesis via immunomodulation [77].

Various other inorganic nanomaterials, including nanocrystals, nanotubes and nanowires have been designed as multi-potent devices in the field of theranostics. Of these, carbon nanotubes (CNT), a tubular structure composed of sheets of benzene rings, have various properties that make it an excellent molecule not only for diagnostic purposes, but also therapeutic in the form of phototherapy as well as cellular therapy [78-80]. CNTs have been used as a contrast agent in PET scans for molecular imaging and were found to have 100-times efficacy when compared to traditional contrasts [81].

Magnetic Carbon nanotubes [MWCNTs] have been shown to have

a satisfactory lymphatic distribution and have also been conjugated to chemotherapeutic agents to provide treatment of lymphatic melanoma metastasis and SNLB [82]. There is also a great scope for use as drug delivery systems, photodynamic therapy and gene therapy.

Conclusion

Melanoma still remains a killer disease. As more advances are made in understanding its molecular profile and pathogenesis, more scope for early diagnostic and effective treatment presides. Nanotechnology has the potential to improve both the diagnosis and treatment of this disease. Combining nanoparticles with new advances in diagnosis, in addition to the biological and chemical therapies has immense scope and potential. Nanotechnology has already shown to be efficient in delivering anticancer drugs, improving efficacy and reducing side effects.

Nanoparticles are successfully used to carry imaging agents for melanoma detection and specific mutation silencing. Exciting and encouraging research is being published on a daily basis in the field of nano-oncology and there is successful translation into clinical

practice. More information is however required on the long-term safety and toxicity of these applications and more work are required on combining the most promising therapies such as ipilimumab with nanoparticles. Ipilimumab, marketed as Yervoy, is a drug used for the treatment of cancer. It has been approved in the U.S. by FDA for the treatment of melanoma since 2011. More clinical studies underway at present are summarized in Table 2.

One thing is sure, nanotechnology is a promising filed, providing hope for the future in the worldwide quest for curing melanoma. Nanoparticle by nanoparticle may we achieve this goal.

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