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RECENT DEVELOPMENTS IN METASTATIC MELANOMA RESEARCH

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ABSTRACT

Melanoma is a type of cancer that originates from pigmented cells called melanocytes. Staging of melanoma is based on the primary tumour thickness, ulceration, lymph node and distant metastases. Location of metastasis and level of LDH (lactic acid dehydrogenase) are the key predictors helping in survival of patients with stage IV melanoma. Treatment to metastatic melanoma primarily targets BRAF, VEGF (vascular endothelial growth factor) receptor, PGDF (platelet derived growth factor) receptor, Raf kinases, MEK, and mTOR. The risk factors include the number and appearance of melanocytic nevi, family history, chronic sun exposure, socioeconomic status and certain phenotypic characteristics. The conventional therapy approaches such as immunotherapy by the use of drugs, vaccines, surgery, chemotherapy, combinational chemotherapy and radiation therapy although offers therapeutic benefits in prevention of cancer; the survival rate of the patients was comparatively less. Unfortunately, resistance to chemotherapy is a challenge in the treatment of metastatic melanoma. Thus, targeted therapy by the use of drugs such as Vemurafenib, Trametinib, nanoparticles, siRNA, dendrimers, liposomes etc. are proven to be safe and effective in the treatment of cancer and also improves the drug solubility, specificity and stability, while simultaneously decreasing the adverse effects. Nano drugs can kill tumour cells directly or can act as carriers for chemotherapy and gene therapy. siRNA therapy alone or in combination with chemotherapeutic drug(s) is a promising strategy for cancer treatment, with reduced toxicity, minimal dose requirement and efficacious when compared to that of conventional therapies.

INTRODUCTION

Metastatic melanoma is derived from melanocytes and is characterised as malignant tumour. Even though it constitutes for 4 per cent of skin cancer it leads to 79 per cent of deaths related to skin cancer. As per American Cancer Society report, the incidence of melanoma cases was estimated to be 97,000 while mortality rates were approximately 7,300 in both males and females(1). Figure 1 shows the drugs that have been approved in recent years by USFDA in treating metastatic melanoma. Melanoma treatment mainly involves two limitations: one with adverse effects resulting in toxicity of the GI and skin, immune responses and loss of tumour specificity, and the other with diminished efficacy due to immune resistance, chemotherapy and targeted therapy(2). Melanoma needs to be treated with radiotherapy, laser therapy, immunotherapy,

etc. when metastasised, although surgical intervention was possible if detected early(3).

Tumour, node and metastasis (TNM) staging of malignant melanoma

TNM staging categorizes the melanoma into four stages depending on the involvement of lymph nodes, the site and distant metastasis as depicted in Table 1 (4)

RISK FACTORS: Melanoma is a multifactorial disease. The number and shape of melanocytic nevi, heredity, exposure to the sun, phenotypic characteristics such as red hair, fair skin, light eyes, sensitivity to the sun, family atypical mole / melanoma syndrome (FAMM) and high socioeconomic status are the most important factors for the development of melanoma(5). Figure 2 shows the two distinct biological pathways that trigger metastasis in melanoma, one being chronic sun exposure characterized by NRAS mutation and other being intermittent sun exposure linked to the nevus prone pathway associated with BRAF pathway(6).

CONVENTIONAL THERAPIES TO TREAT MELANOMA

Chemotherapy, drug therapy, immunotherapy, vaccines, radiation therapy etc. are few conventional methods used in the diagnosis and management of melanoma and its metastasis.

Drug therapy: Drug therapy for melanoma has advanced considerably in recent years. Stage III and Stage IV patients used to have about seven months of overall survival, whereas patients can now live for many years. Two important classes of drug treatments for metastatic melanoma include oral targeted therapy for tumour patients with BRAF mutation and intravenous immune modulators that support an individual's own immune response to tumours(4). The drugs used to treat melanoma acts commonly on MEK and BRAF pathways. The drugs along with the percentage of tumour shrinkage and duration of therapeutic benefit are been discussed in table 2.

Surgical resection: The primary treatment for localized melanoma involves removal of tumour, peripheral healthy tissue surgically and the biopsy of sentinel lymph node is done in patients with tumour size larger than 0.8 mm or smaller than this but ulcerated (stage pT1b or greater). When melanoma cells in sentinel lymph nodes are detected then the remaining lymph nodes in the region are sometimes removed. In some cases, metastatic tumours can also be removed by surgery but are not successful(7).

Chemotherapy: The clinical responses showed improvement by combinations of chemotherapy, but no improvement was shown in overall survival (OS). In chemotherapy the drug resistance is mainly caused due to apoptosis resistance. In patients with advanced melanoma the response rates were found to be between 10 and 20 per cent with single agent chemotherapy. Temozolomide (TMZ), cisplatin,dacarbazine, docetaxel carboplatin, vinblastine, fotemustine, and paclitaxel are the most commonly used chemotherapy agents. In metastatic melanoma,Dacarbazine is used as single-agent therapy most frequently. For monotherapyin melanoma, vinca alkaloids have been used for single agent activity. Vindesine (a vinblastine derivative) and Docetaxel are used most frequently(8).

Combination chemotherapy: Dacarbazine and additional chemotherapeutic agents like nitrosoureas or platinum-based compounds were used in early research stages of combination therapy. These findings lead to development of multidrug chemotherapy regimens.Dacarbazine/ vinblastine / Cisplatin and cisplatin / carmustine (BCNU, a nitrosourea) /tamoxifene are probably the best known and commonly used of these combinations. Cytokine-based therapy, sometimes called bio chemotherapy, is tested in combination chemotherapy or conjunction with single-agent. Other multidrug chemotherapy regimens alone or dacarbazine alone has shown better patient survival compared to IFN-a and IL-2 when tested with dacarbazine, which showed no improvement in survival rate. Compare to single agent therapy combination therapies showed better response rate and increased toxicity. Consequently, the welfare of the patients receiving combination chemotherapy are still challenging(3).

Immunotherapy Vaccines: By injecting subcellular fractions of allogeneic or autologous melanoma cells into melanoma subjects for vaccination studies, which was then inactivated by pharmacological treatments or radiation therapy with the assumption that tumour cells are expressedas recognised by the immune system. Berd et al. in their studies observed the response of T cell by vaccine administration which is been depicted in table 3(9). Figure 3 shows the steps involved in immunotherapy such as selection and activation of tumour cells (IL-2) and infusion into the tumour site.

A. **Oncolytic viral therapy:** Subgroup analysis of the OPTiM trial showed a significantly higher lasting response rate of 33 per cent in patients with stage IIIB / C and significantly improved overall survival with TVEC for stage IIIB / CorIVM disease. Conry et al(10) in their studies demonstrated that Imlygic was able to treat BRAF stage IIIB / C and IV disease (+/-) when supplied with Talimogenelaherparepvec (T-VEC). T-VEC C ipilimumab mainly increased flu-like symptoms with no unexpected adverse events or deaths associated with the procedure. BCG vaccine contains oncolytic gene that helps in malignant melanoma treatment.Pembrolizumab is a monoclonal IgG4 antibody directed against PD-1 and is recommended for metastatic melanoma treatment and several other malignancies. These findings suggest that oncolyticvirotherapy can improve the effectiveness of anti-PD1 therapy by altering the tumour microenvironment.

ADVANCED THERAPIES IN THE TREATMENT OF METASTATIC MELANOMA

1. **Targeted therapy:** Melanoma is caused by genetic events such as mutation, gene amplification, and gene deletion which alters the growth cycle and normal cell survival rate. Genetic defects further affect the significant signalling pathway as their dependence on the cycle of survival and growth is called oncogene addiction. Thus, the targeted therapy which usually contains antibodies or small molecular inhibitors aims at inhibiting the signalling pathway that would lead to cancer progression. Another main advantage is that it kills only cancer cells without any impact on peripheral healthy cells. Few categories of targeted therapy have been discussed below.

A. **BRAF inhibitors:** BRAF is a primary serine – threonine kinase signalling pathway using MAPK.For example,Vemurafenib andDabrafenib are FDA-approved BRAF-mutant inhibitors. In monotherapy, various clinical trials are on-going with vemurafenib and dabrafenib, combined with immunotherapy, chemotherapy and other targeted therapies.

B. **MEK inhibitors:** Trametinib, a MEK1/2 anti-cancer drug has been approved by the FDA in treating metastatic melanomas. Inhibition of growth factor-mediated cell signalling and decline in proliferation of tumour cells and inhibition of growth factor-mediated cell signalling is caused by blockage of MEK 1/2. In a clinical trial, trametinib and dabrafenib combination therapy was found to be effective against metastatic melanoma with BRAF mutation and was approved by FDA in 2014. Trametinib and dabrafenib combination in radiotherapy, immunotherapy, and targeted therapy, several clinical trials are on-going. Combination therapy comprising Vemurafenib and Cobimetinib, an oral selective MEK inhibitor combination therapy are FDA approved(11,12).

C. **PI3K-AKT-mTOR pathway inhibitors:** Growth of tumour is influenced by mTOR . PI-103, a PI3 K inhibitor with rapamycin is an effective mTOR inhibitor. Higher rate of apoptosis was found in PI3K-AKT compared to MEK or BRAF inhibitors. A clinical trial involving everolimus and temsirolimus (mTOR inhibitors) with BRAF inhibitor are in progress(13).

2. Application of nanotechnology as a novel therapy for malignant melanoma

Recent developments in nanotechnology for treating metastatic melanoma are emerging as it reduces the extent of problems that other drug therapies have, such as lack of cell targeting, drug solubility and stability, while reducing adverse effects. The ability to target tumour with greater precision is what differentiates nanotechnology(14).

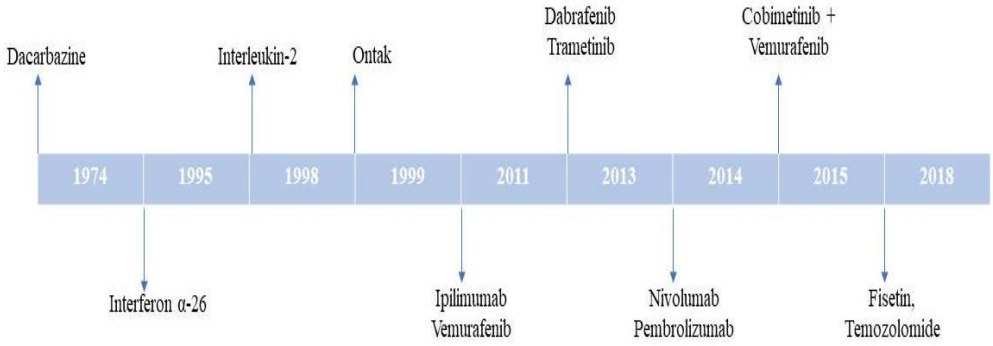

Figure 1: USFDA approved therapy

Table 1: TNM staging

Stage	Thickness of tumour	Involvement of lymph nodes	Distant metastasis
I	<2.0mm thick	-	-
II	1–2.0mm thick	-	-
III	Any thickness	-	-
IV	Any thickness	Involved	Yes

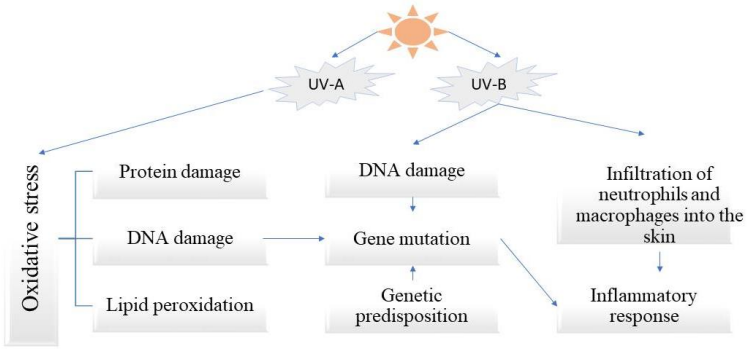

Figure 2: UV induced melanoma

Table 2: Drugs used in metastatic melanoma treatment

Drug	Mechanism of action of drug	Percentage of tumour shrinkage	Duration of therapeutic benefit
Dabrafenib + trametinib	Inhibits MEK pathway and BRAF	50% when dabrafenib is taken alone and 70% in combination	9 months and up to 12 months in combination
Ipilimumab	Anti CTLA-4 antibody	15%	3 months
Nivolumab	Anti-PD-1 antibody	40%	7 months
Pembrolizumab	Anti-PD-1 antibody	30%	5 months
Vemurafenib + cobimetinib	Inhibits BRAF and MEK pathway	50% on taking vemurafenib alone and 70% shrinkage of the tumour on combination	Up to 7 months if taking vemurafenib alone and 12 months in combination

Table 3: Vaccines in the treatment of metastatic melanoma

Vaccines	T-cell response
Canvaxin	-
Autologous tumour-derived HSPPC96	48%
M-VAX	-
Autologous melanoma cells engineered to secrete GM-CSF by adenoviral-mediated gene transfer	-
VacciniavirusexpressingB-71	83%
Autologoustumour-derivedHSPPC96	32%

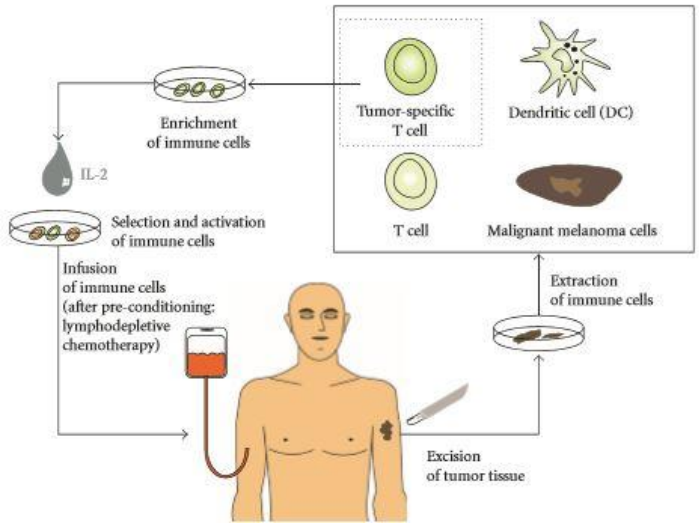

Figure 3: Immunotherapy in metastatic melanoma

Table 4: Nanoparticles as carriers of drug

Type of nanoparticles	Cell	Effect	Mechanism
Anionic gamma-Fe_2O_3 nanoparticles	Epidermal melanoma (SK-Mel-28)	Apoptotic effect	Induce DNA fragmentation
Cerium oxide polymer coated NPs	A-375	Anti-invasive, Cytotoxic and pro-apoptotic	Inhibition of caveolin-1 expression and pro-oxidant
Realgar NPs	BOWES and A-375	Autophagy and apoptotic effect	Induce damage to DNA, redox stress and phosphorylation of proteins
Selenium NPs	A-375	Apoptotic effect	Induce oxidative stress, dysfunction of mitochondrial function
Single-walled carbon nanohorns	HT144	Anti-proliferative	Growth inhibition, anti-proliferative, anti-mitotic
ZnO:AgNPs	CRMM-1 (Conjunctival melanoma cell line)	Cytotoxicity	ROS generation and peroxidation of lipids

Nanoparticles: Nanoparticles (NPs) reduce degradation with the ability to trap drugs, improve the solubility of poorly water-soluble drugs and enable higher cell targeting specificity. There are essentially two ways NPs can target tumour: active and passive targeting. The passive targeting of tumour is based on increased permeation and retention of tumour-specific cells that allows NPs to accumulate at high concentrations within tumours. Active targeting is a specific technique which functions by NPs binding to MM cells

expressing specific receptors or overexpress receptors compared to healthy cells, followed by internalization of the drug with receptor-mediated endocytosis(15). The nanoparticles possessing direct killing effect act by either of the ways; DNA damage, disruption of cell membrane and oxidative stress.

- **Nanoparticles as carriers**

i. Nanoparticles as drug carriers

Zinc oxide, selenium, dendrimers, realgar, polymers etc. act as carrier for delivering the drug at the target site. These drug encapsulated nanoparticles act by apoptosis, cytotoxicity effect against cancer cells, autophagy etc. The mechanism of action of the nanoparticles along with the cells they interact with and the effect on tumour is been enlisted in table 4(16).

Nano particles as carrier of genes

Positively charged nanoparticles composed of bovine serum albumin (BSA) encapsulating siRNA demonstrated higher rate of uptake by tumour and encapsulation efficiency. Thus, the therapy was found to be effective against B16 lung metastasis(17). Polyethylenimine (PEI) nanoparticles modified with stearic acid (StA) encapsulating STAT3- siRNA can be transported efficiently in the cytoplasm of B16 melanoma cells. STAT-3 and VEGF levels substantially decreased, the development and metastasis of tumour was inhibited(18).

Photodynamic therapy for melanoma: PAMAM copolymer (Polyamidoamine) covalently bound to photosensitizer chlorines e6 is an effective photodynamic therapy for malignant melanoma. NPs with RGD targeting moieties enhance the curative effect and cellular uptake. A gold nanoshell could produce heat for PTT and produce singlet oxygen for PDT under near-infrared light irradiation, which effectively inhibited the growth of melanoma cells(19).

Gene therapy using siRNA: siRNA based therapy is a promising strategy for melanoma treatment. The combination of chemotherapeutic drugs with siRNA results in reduction of dose and improved efficacy. Currently, chemotherapies in combination with siRNA given via oral, IV and IM routes to treat melanoma have been developed.Anti-metastatic siRNA loaded in Methoxy-polyethyleneglycol derived polycaprolactonemicelles which are functionally modified with cytoplasm responsive peptide known to suppress melanoma development as it inhibited the pulmonary metastasis corresponding to B16F10 melanoma and also affects VEGF(20). MNs fabricated from dextran, hyaluronic acid and polyvinylpyrrolidone biocompatible vehicles for delivering STAT3 PEI/siRNA demonstrated improved anti-proliferative effect, cellular uptake and gene-silencing efficacy in B16F10 melanoma (21).Ruan et al in their research developed a fusion peptide SPACE-EGF carrier to enhance the penetration of siRNAs into the skin and cells for targeting B-16 cells and apoptosis; the tendency of suppression of tumour growth was consistent(22).

Liposomes: Hyaluronic acid coated liposomes encapsulatingEugenol and Dacarbazine allow for successful targeting of metastatic melanoma.Eugenol has *in vitro* and *in vivo* melanoma activity which is anti-proliferative and apoptosis-inducing. Dacarbazine is an alkylating agent and finds use in melanoma combination therapy. Hyaluronic acid has affinity to receptors of CD44, which is overexpressed in most cancer cells(23). Ad-PEDF anti-angiogenic gene therapy; a recombinant PEDF adenovirus loaded into cationic liposomes by Ad-PEDF has an inhibitory effect on B16-F10 melanoma cells and showed marked effect on tumour suppression and apoptosis. Ad-PEDF liposome was found to be more active compared to Ad-PEDF (uncoated) cells in order to prevent in vivo pulmonary metastases(24).

Dendrimers: Dendrimersare highly branched macromolecules that offer profound characteristics such as unit polydispersity, surface chemistry, unique chemistry and structure, controlled size and was first synthesised by Vogtle in 19781.Polyamidoamine (PAMAM) dendrimersare non-immunogenic and water soluble. PAMAM G5 and PAMAM G2.5 as carboxylic acid surface moieties were used as core and shell materials in synthesising G5G2.5 tectodendrimers. The evaluation studies

showed that the uptake of G5G2.5 by SK-MEL-28 cells was high and tactodendrimer consisting G5G2.5 also demonstrated cytotoxic effect on SK-MEL-28 cells in a concentrationdependent and selective manner(25). Temozolomide (TMZ) encapsulated PAMAM dendrimers are applicable in targeting A375, a human melanoma cell line *in vitro*. This drug delivery system increased the sensitivity of human melanoma cells, A375 TMZ, thus can be used as a novel strategy in the treatment and target of metastatic melanoma(26).

CONCLUSION AND FUTURE PROSPECTIVES

The treatment of metastatic melanoma is complicated with occurrence rates being higher. Nanotechnology has a bright future in treating malignant melanoma. Certain molecules are under preclinical trial investigation at present while few are in clinical trials. Thus, there is a hope for improved therapeutic efficacy. For malignant melanoma, chemotherapeutic drugs include dacarbazine (DTIC), temozolomide (TMZ), paclitaxel, and platinum compounds. Nevertheless, a single agent's overall response rate is less than 20 per cent, and these agents have many side effects. Chemotherapeutic resistance is a major problem in the treatment of metastatic melanoma. At present, advances in immunotherapy and molecular targeting therapy have improved prospects for patients.Unfortunately for all patients these therapies aren't successful. Still, the effects of further treatment are negatively influenced by drug resistance and immune-related side-effects. Recently, rapid developments in nanotechnology have been promising for cancer treatment. Nano drugs can directly kill tumour cells, or serve as carriers for chemotherapy and gene therapy. Photodynamic therapy (PDT) and photo thermal therapy (PTT) comprising of nanoparticles have gained considerable importance in the treatment of metastatic melanoma.

Conflict of Interest Statement: Authors have no conflict of interest to declare.

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