Recent Changes and Innovations in Melanoma Treatment: A Review

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ABSTRACT Malignant melanoma is one of the most extensively studied diseases in the last few decades. The outcome of these studies and the treatment changes that followed have dramatically altered the landscape of not only melanoma therapy, but all solid tumors. In this review we presented the recent advances of surgical and adjuvant management of patients with cutaneous malignant melanoma. This review focuses on stage III melanoma since this stage of disease requires surgical treatment as well as adjuvant therapy.

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MELANOMA

Cutaneous malignant melanoma (CMM) is a cancer disease that develops from uncontrolled proliferation of melanocytes. Melanocytes are mostly present in the skin, at the basal layer of the epidermis, active as pigment-producing cells. The pigment, called melanin, is created in the melanosomes and spread to neighboring keratinocytes. The melanin serves as a DNA protector from ultraviolet radiation (UV) exposure, since UV exposure can result in DNA damage by formation of new dimers. This accumulate damage can result in an uncontrolled deviation of melanocytes. In sun exposed skin we can see melanocytes proliferation, called atypical melanocytic hyperplasia, which over time may develop to become CMM.

EPIDEMIOLOGY

In the United States, CMM is the fifth leading cancer in men and women [1]. According to the Israeli cancer registry [2], there were 1846 new patients diagnosed with CMM in 2016, which is about 6% of cancer patients diagnosed in 2016. Of those, 43% were diagnosed with melanoma in situ, 44% with localized disease, 6% with regional melanoma and 3% with metastatic melanoma. 93% of patients were Jewish, 2% were Arabic and 5% unknown ethnicity.

Due to the majority of patients with melanoma in Israel being Jewish, the following data only includes information about the Jewish population in Israel.

The highest incidence of invasive CMM among Jewish ethnic subgroups standardized by age for 100,000 was American-Europeans (18.5 men, 13.4 women) and Israeli born (16.2 men, 11.6 women). Lower rates were observed in Asians (5.0 men, 3.9 women) and Africans (2.4 men, 4. in women).

Only 1% of the new invasive melanoma were found in people younger than 25 years of age. The morbidity was most common in people older than 75 years of age, while the average age of diagnosis in men was 66.9 and in women 63.9. Assuming life expectancy of 80 years, a Jewish man has a 3.5% chance to get CMM during his lifetime.

Between 2009 and 2016, the morbidity rates in Jewish men showed a slight but statistically significant increase (annual percent change +0.86%, $P < 0.05$). In Jewish women the morbidity rates were stable during these years.

According to the new global cancer data that was published in 2018 [3], of the countries with the highest incidence rates of melanoma, Israel was 27th, while Australia was at the top of the list.

STAGING

CMM staging is classified by TNM classification of malignant tumors. In January 2018, the American Joint Committee on Cancer introduced the Eighth Edition Melanoma Staging System [4], which includes multiple changes from the Seventh Edition.

T-tumor

T-stage nomenclature, and T-stage categorization by Breslow depth, were updated. The second decimal point was omitted from Breslow measurements and tumor mitosis rate (to identify T1b lesions) is no longer used as a staging criterion [Table 1].

N-nodal

The word micrometastases was replaced with the term "clinically occult" and macrometastases was replaced with "clinically detected". That might seem only a semantic change, but this change testifies how important the clinical evaluation is in the management of melanoma, especially in the evaluation of lymph nodes involvement [Table 2].

M-metastases

Elevated lactate dehydrogenase (LDH) is no longer a category (M1c), but an addition to any M-level. Normal LDH would be characterized as (0) and elevated LDH as (1). A new M category was added, M1d, which refer to metastasis in the brain [Table 3].

An update of the diagnosis and management of melanoma was published in 2018 in *Plastic and Reconstructive Surgery* [5]. The authors included the important changes at melanoma stag-

Table 1. T-tumor

	Seventh edition	Eight edition
Tx	Primary tumor cannot be assessed (e.g., curettage or regression)	
T0	No evidence of primary tumor	
Tis	Melanoma in situ	
T1	**≤ 1.0 mm**	
T1a	**Without ulceration and mitosis < 1/mm²**	**< 0.8 mm without ulceration**
T1b	**With ulceration or mitosis ≥ 1/mm²**	**0.8–1.0 mm without ulceration or ≤ 1.0 mm with ulceration**
T2	**1.01–2.0 mm**	**1.1–2.0 mm**
T2a	Without ulceration	
T2b	With ulceration	
T3	**2.01–4.0 mm**	**2.1–4.0 mm**
T3a	Without ulceration	
T3b	With ulceration	
T4	**4.0 mm**	
T4a	Without ulceration	
T4b	With ulceration	

Bold signifies the changes in the eighth edition

ing. According to the article one of the greatest impacts of the American Joint Committee on Cancer Eighth Edition is on stage III disease. For example, in the seventh edition, stage IIIa and stage IIIb were defined by the status of regional lymph nodes and primary tumor ulceration. Many thick, non-ulcerated tumors with limited micrometastases were previously staged IIIa but have now been reassigned to stage IIIc. Therefore, survival in stage IIIa and IIIb has improved, as these two stages are now restricted to thinner primary tumors.

By better staging the eighth edition allows to have more exact research, and following that, better management and treatment compared with the seventh edition stage [Table 4].

DIAGNOSIS

It is well known that complete excision of a tumor is the most important step toward healing. Skin biopsy is a necessary procedure not only to diagnose CMM but also to achieve an appropriate surgical excision.

Biopsy

The optimal biopsy technique in a lesion suspected as CMM is an excisional biopsy [6] because partial biopsy techniques such as shave, punch, and incisional are significantly more likely to have positive margins after definitive wide local excision and more frequently require early reoperation. Nevertheless, the biopsy technique does not influence the incidence

Table 2. N-nodes

		Seventh edition	Eight edition
Nx	Regional nodes cannot be assessed		
N0	No regional metastases detected		
N1	**One node**		
	N1a	**Micrometastasis**	One node, **clinically occult**, without satellites, local recurrence or in transit metastases
	N1b	**Macrometastasis**	One node, **clinically detected**, without satellites, local recurrence or in transit metastases
	N1c	-	No nodes, with satellites, local recurrence or in transit metastases
N2	**2-3 nodes**		
	N2a	**Micrometastasis**	2–3 nodes, **clinically occult**, without satellites, local recurrence or in transit metastases
	N2b	**Macrometastasis**	2–3 nodes, **clinically detected**, without satellites, local recurrence or in transit metastases
	N2c	In-transit metastasis or satellites without metastatic nodules	One node, clinically occult or detected with satellites, local recurrence or in transit metastases
N3	**4 or more nodes**		
	4 or more nodes, matted nodes, or in-transit metastasis or satellites with metastatic nodules		• 4 or more nodes, all clinically occult, without satellites, local recurrence or in transit metastases • 4 or more nodes, at least 1 node clinically detected or matted, without satellites, local recurrence or in transit metastases • 2 or more nodes, clinically occult or detected with satellites, local recurrence or in transit metastases > 1 node

Bold signifies the changes in the eighth edition

Table 3. M-metastases

	Seventh edition	Eight edition
M0	No detectable evidence of distant metastases	
M1a	Metastases to skin, subcutaneous or distant lymph nodes	**Any M level would be (0) if LDH is normal and (1) if LDH is elevated**
M1b	Metastases to lung	
M1c	Metastases to visceral sites or distant metastases to any site, combined with an elevated serum LDH	
M1d	**-**	**Metastasis to brain**

LDH = lactate dehydrogenase
Bold signifies the changes in the eighth edition

of detected sentinel nodes or their amount, the overall survival or disease-free survival. If excisional biopsy is not possible, punch biopsy is also acceptable while shave biopsies are least reliable as these tend to miss the tumor depth [7]. The biopsy of a lesion suspected as CMM should be with 1-3 mm clinically negative margins [7].

The information that a pathology report should contain, according to the AJCC [8], includes size of specimen, tumor thickness (Breslow), ulceration, dermal mitotic rate, peripheral and deep margin status, and microsatellitosis.

Dermoscopy diagnosis

Biopsy is the only definitive diagnostic tool for melanoma. However, biopsy must be used only in cases with a reasonable level of suspicion, as unnecessary biopsies may leave scars, and in some cases could cause wound infection or other complications.

Dermoscopy is a microscopy-based tool to improve noninvasive diagnostic of skin lesions based on color and structure analysis. The technique is based on the penetration of polarized light into the dermis, thus allowing visualization of structures in the dermoepidermal junction and superficial dermis rather than on the surface only. In the last few years, dermoscopy use has become more common, and recent research shows that use of dermoscopy and detection algorithms by primary care physicians can enhance assessment of clinically suspicious lesions compared with that of naked eye examinations [9]. Of course this evaluation is operator dependent and cannot take the place of histopathologic diagnosis. However, a great effort is invested in numerous technologies that will hopefully improve noninvasive diagnosis of CMM [10].

Combined adjuvant treatment with B-RAF inhibitors and MEK inhibitors prolongs relapse-free survival in patients with stage III melanoma

SURGICAL TREATMENT

Wide local excision

After the pathological report confirms the diagnosis of melanoma, a wide excision should be performed. The goals of a wide excision are to ensure complete removal of the lesion and achieve histologically clear margins, thus reducing the risk of local recurrence. Surgical margins should be based on tumor thickness. The recommended margins for melanoma are based on prospective randomized controlled trials and are the strongest category of recommendation according to the *Journal Academy of Dermatology* guidelines [11].

For a tumor thickness less than 1 mm the surgical margin should be 1 cm [12], for a tumor thickness between 1 and 2 mm the surgical margin should be 1–2 cm [13], and for a tumor thickness larger than 2 mm the surgical margin should be 2 cm [14]. In a case of melanoma in situ, wide excision with 0.5–1.0 cm margins is recommended [15]. Melanoma in situ may require greater than 0.5 cm margins to achieve histologically negative margins because of subclinical extension.

Sentinel lymph node biopsy

In many cases, in order to achieve reliable staging and respectively provide the most adequate treatment [16], there might be an indication for a sentinel lymph node biopsy (SLNB), depending on the Breslow thickness. Patients with melanoma of 1 mm thickness or greater (> T1b) should undergo SLNB [17]. SLNB should be discussed with patients with tumor thickness 0.8–1 mm and with selected patients with tumor thickness less than 0.8 mm (T1a) if other adverse features are present [18].

When there is an indication for SLNB, data support performing both wide local excision and SLNB at the same time. The SLNB should be performed first to minimize disruption of the lymphatic channels and to optimize the accuracy of lymphatic mapping to identify the ultimate sentinel node [19]. However, sometimes when the SLNB and primary tumor are in close proximity, radioactive gamma radiation from the injection site may interfere with finding the SLNB and performing a wide excision first may improve SLN detection [20].

Regional complete lymph node dissection (CLND)

For many years, the next recommended step after a positive lymph node biopsy was regional complete lymph node dissection (CLND). The hypothesis that led to this practice was that regional lymph nodes act either as active filters to the disease spread [21] or as passive indicators of the chances of distant metastases [22]. However, in melanoma, no great evidence supports the value in removing clinically negative nodes.

Table 4. Staging

Stage group	T classification	N classification	M classification
0	Tis	N0	M0
IA	T1a	N0	M0
IB	T1b	N0	M0
	T2a	N0	M0
IIA	T2b	N0	M0
	T3a	N0	M0
IIB	T3b	N0	M0
	T4a	N0	M0
IIC	T4b	N0	M0
IIIA	T1-2a	N1a	M0
	T1-2a	N2a	M0
IIIB	T0	N1b-c	M0
	T1-2a	N1b-c	M0
	T1-2a	N2b	M0
	T2b-3a	N1a-2b	M0
IIIC	T0	N2b-c	M0
	T0	N3b-c	M0
	T1a-3a	N2c-3c	M0
	T3b-4a	Any N	M0
	T4b	T1a-2c	M0
IIID	T4b	N3a-c	M0
IV	Any T	Any N	M1

Two prospective, randomized, clinical trials that were published in 2016 and 2017 led to a revolution in the management of patients with a positive SLNB. The results of the DeCOG-SLT [23] and the MSLT-II [24] trials showed that an immediate regional CLND after a positive SLNB without clinical evidence of lymph node metastases does not increase melanoma-specific survival.

The MSLT-II trial included 1934 patients with sentinel node metastases, stage 3 melanoma detected by standard pathological assessment or RT-PCR assay. Most of the patients had low volume disease with 0–3 positive sentinel lymph nodes. The participants were randomly assigned to complete lymph node dissection (CLND group) or nodal observation with ultrasonography (observation group).

The mean melanoma specific survival was similar in the CLND group and the observation group (86 ± 1.3% and 86 ± 1.2%, respectively) at a median follow-up of 43 months. Although the rate of disease-free survival, based on the rate of disease control in the regional nodes during 3 years, was slightly higher in the CLND group at 3 years, it did not make a valuable difference in melanoma specific survival.

Other from those analyses, some sub-analyses were conducted to evaluate the optional benefit of CLND over observation. One of the subanalyses observed separately the melanoma specific survival between the group who had positive LNB detected by standard pathological assessment and those who were detected by RT-PCR. This subanalysis did not reveal any subgroup that derived a significant melanoma specific survival benefit from CLND. These data are important indicators that show that the characteristics of the sentinel lymph node do not influence the surgical management.

As expected, lymphedema, which has a high morbidity rate [25], was observed in 24.1% of the patients in the CLND group and in 6.3% of those in the observation group.

These results have proved that immediate CLND does not increase melanoma-specific survival among patients with stage 3 melanoma and positive SLNB; however, it does increase the number of patients with a complication of lymphedema. The DeCOG-SLT trial, which examined the same questions, came to similar conclusions.

As long as there is no evidence of lymphatic metastases on examination or according to ultrasound imaging, there is no value in regional complete lymph node dissection, including patients with positive sentinel lymph node biopsy

ADJUVANT TREATMENT

Cancer immunotherapy is one of the most important recent developments in the world of medicine. Many different methods of activating the immune system against cancer cells have been researched; from intravenous immunoglobulin therapy [26] to targeted therapy. The idea behind these therapies is to use a patient's immune system to cure his or her own disease. The therapy targets immune checkpoints and by doing so restores immune system function against cancer cells, which were identified as 'self' until then. Before the approval of the first check point inhibitor in 2011, the median overall survival (OS) of patients with disseminated melanoma was 9 months. According to current data, the median OS is 2 years or more [27]. Between the years 2011 to 2019, the U.S. Food and Drug Administration (FDA) has approved nine new drugs for the treatment of melanoma. Most of these drugs have improved overall survival. Primarily the use of these drugs was for unresectable melanoma. Once they proved to be successful, the next step was to evaluate these drugs as adjuvant treatments. We will review some of the innovative treatments used as adjuvant therapy for patients with stage III melanoma through recent years, and present the current practice in management of these patients.

Interleukin-2

High dose Interleukin-2 (IL-2) was the first immunotherapy agent approved for the treatment of unresectable or metastatic melanoma. IL-2 is not a checkpoint inhibitor, it is a cytokine that proliferates T cells and promotes their antitumor activity. The efficacy of this treatment has been proven by complete responses seen in some patients, some of these responses continuing for over a decade and indicated cure [28]. The drawback of this treatment is severe toxicity associated with its use. Due to its toxic profiling, this therapy is not a common practice anymore. However it is important since it has opened a new realm of treatments for melanoma.

Anti-cytotoxic T-lymphocyte-associated protein 4

Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) is an immune checkpoint that downregulates the immune response. Anti-CTLA4 therapy, a human IgG1 monoclonal antibody, blocks the interaction of the negative regulator CTLA4 on effector T cells. Anti-CTLA4 has shown improved survival compared with chemotherapy in patients with unresectable melanoma [29].

In 2019 results of phase 3 randomized controlled trial were published [30]. The aim of the trial was to assess anti-CTLA4 as an adjuvant therapy for patients with stage III melanoma at high risk of recurrence. In this trial patients were randomly assigned to receive anti-CTLA4 or a placebo, while the primary endpoint was recurrence-free survival. At a median follow-up of 5.3 years, recurrence-free survival, distant metastasis-free survival, and overall survival were each significantly prolonged in the anti-CTLA4 group compared with the placebo group. The most common serious adverse events in the anti-CTLA4 group were gastrointestinal and hepatic. The dose and duration of anti-CTLA4 had a high toxic profile, which led to more research for an ideal effective treatment with a low toxic profile.

Adjuvant treatment with PD-1 antibody prolongs recurrence-free survival in patients with stage III melanoma

The anti-CTLA4 trial included patients who had undergone a complete lymphadenectomy. There is no indication for complete regional lymph nodes dissection in patients with stage III melanoma even with positive SLNB, unless there is a clinical or imaging proof for nodes metastases. Continued research is needed in which the patients are treated by the most relevant recommendations.

ANTI-PD-1/PD-L1

Programmed cell death protein 1 (PD-1) has a role in the regulation of the immune system's repose to the human body cells by down regulating the response and suppressing T cell inflammatory activity. This protein acts in the prevention of autoimmune diseases but it also prevents the immune system from reacting to cancer cells as it recognizes them as self. The immune checkpoint PD-1 binds two ligand; PD-L1 and PD-L2.

The PD-1 inhibitor, a human IgG4 monoclonal antibody, has been found to prolong progression-free and overall survival in patients with unresectable and metastatic melanoma, regardless of PD-L1 expression level [31].

In 2019, results of a new trial [32] were published that showed that among patients undergoing resection of stage III or IV melanoma, adjuvant therapy with PD-1 inhibitor results in a significantly longer recurrence-free survival and a lower rate of adverse events than therapy with anti-CTLA4.

A phase 3 double blind trial was published in 2018 [33] and its goal was to evaluate PD-1 inhibitor as adjuvant therapy in patients with resected, high-risk stage III melanoma. In that trial the patients were treated by a PD-1 inhibitor or a placebo and the primary endpoint was recurrence-free survival. At a median follow-up of 15 months, PD-1 inhibitor was associated with significantly longer recurrence-free survival than placebo (75.4% vs. 61%, $P < 0.001$).

PD-1 inhibitor was consistently effective in patients with PD-L1 negative tumors and in those with undetermined tumor PD-L1 expression. Serious adverse events related to the trial regimen were reported in approximately 15% of the PD-1 group and in 3.4% in the placebo group. The most common adverse events were immune related, such as endocrine disorders, sarcoidosis, and colitis. This trial also included patients who underwent a complete lymphadenectomy, which opens a door to a continuing research.

B-RAF and MEK inhibitors

A cell deviation, differentiation, and secretion are effected by signaling pathways, an important one being the MAP kinase pathway. B-RAF and MEK are members of the signal transduction protein kinases that regulates the MAP kinase pathways. Oncogenic mutations in B-RAF are found in approximately 40% of patients with cutaneous melanoma and activate the MAP kinase pathway [34].

B-RAF inhibitors have shown efficacy as a monotherapy in patients with unresectable and metastatic melanoma with B-RAF mutations [35]. Resistance to B-RAF inhibitors developed quickly, which led to the combination of a B-RAF inhibitor with an MEK inhibitor. This treatment combination resulted in a significant delay in the emergence of resistance with a longer overall-free survival and recurrence-free survival than with B-RAF inhibitors alone and without increased toxicity [36]. With the understanding of this treatment value for unresectable melanoma, trials were conducted to determine whether adjuvant B-RAF and MEK inhibitors improved outcomes in patients with resected, stage III melanoma with B-RAF mutation. In 2017, the results of a double-blind, placebo-controlled, phase 3 trial were published [37]. In that

trial, patients with completely resected stage III melanoma with B-RAF mutation received either B-RAF and MEK inhibitors or a placebo for 12 months and the primary endpoint was relapse-free survival. At a median follow-up of 2.8 years, the estimated 3 year rate of relapse-free survival was 58% in the combined therapy group and 39% in the placebo group ($P < 0.001$). The most common adverse events in the combination therapy group were pyrexia, fatigue, and nausea. Serious adverse effects occurred in 36% in the combination therapy group and in 10% in the placebo group.

In 2019, a new study [38] was published. That study analyzed the pooled extended survival data from previous trials. The authors concluded that first-line treatment with B-RAF and MEK inhibitors led to long-term benefit in approximately one-third of the patients who had unresectable or metastatic melanoma with B-RAF mutation.

This trial also included patients with metastatic melanoma who underwent complete lymphadenectomy, which opens the door to continuing research.

LOOKING AHEAD

In the landscape of melanoma, the research and practical changes have changed the nature of the disease as we knew it. A plethora of trials are in progress and new hypotheses are being studied in all aspects of melanoma treatment from the very early diagnosis through surgical treatment and adjuvant therapy.

MOHS

Mohs micrographic surgery (MMS) is an advanced treatment procedure for skin cancer. The surgery relies on the accuracy of a microscope to trace and ensure removal of skin cancer down to its roots. MMS is more commonly used to treat non-melanoma skin cancer rather than melanoma, due to the difficulty in detecting melanoma cells in frozen section. However, recent reports in patients with melanoma in situ treated with MMS compared to wide local excision found that there were no significant differences in the recurrence rate, overall survival, or melanoma-specific survival [39]. With the spreading of MMS techniques for CMM, more patients may enjoy lower morbidity and improved aesthetic outcome.

Combined therapies

Immunotherapy as a targeted therapy has proved to be a treatment that improves prognosis, therefore, many combined therapies are being studied. A randomized controlled trial published in 2019 [40] showed how CTLA-4 combined with PD-1 INHIBITOR prolong overall survival in patients with unresectable advanced melanoma versus anti-CTLA-4 alone. New innovative approaches are being developed that combine immunotherapy with targeted therapy, oncolytic virus, fecal transplantation (microbiome), immuno-pheresis and tumor infiltrated lymphocytes.

CONCLUSIONS

In the near future we might use immunotherapy or targeted therapy as a neo-adjuvant therapy and have a complete resection in tumors that could not be operated before.

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Capsule

Miniproteins against SARS-CoV-2

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is decorated with spikes, and viral entry into cells is initiated when these spikes bind to the host angiotensin-converting enzyme 2 (ACE2) receptor. Many monoclonal antibody therapies in development target the spike proteins. **Cao** et al. designed small, stable proteins that bind tightly to the spike and block it from binding to ACE2. The best designs bind with very high affinity and prevent SARS-CoV-2 infection of mammalian Vero E6 cells. Cryo-electron microscopy showed that the structures of the two most potent inhibitors were nearly identical to the computational models. Unlike antibodies, the miniproteins did not require expression in mammalian cells, and their small size and high stability may allow formulation for direct delivery to the nasal or respiratory system.

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Capsule

Bacteria Breaking and exiting vacuoles

Some bacteria, including *Salmonella* and *Shigella* spp., can take up residence within vacuoles inside mammalian host cells. To gain access to the nutrient-rich host cytosol, the internalized bacteria must break out from their vacuole, but how? **Ellison** and co-authors used super-resolution live-cell imaging and correlative light and electron microscopy to characterize this process. They developed a reporter for the host lipid sphingomyelin, which is normally found on extracellular-facing membranes. The authors found that sphingomyelin became exposed to the cytosol on bacteria-containing vacuoles. This exposure occurred just before the vacuoles ruptured, releasing the bacteria into the cytosol. Sphingomyelin exposure can thus be used as an early indicator of bacterial invasion and potentially more widely as an indicator of membrane damage that threatens the integrity of the cytosol.

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