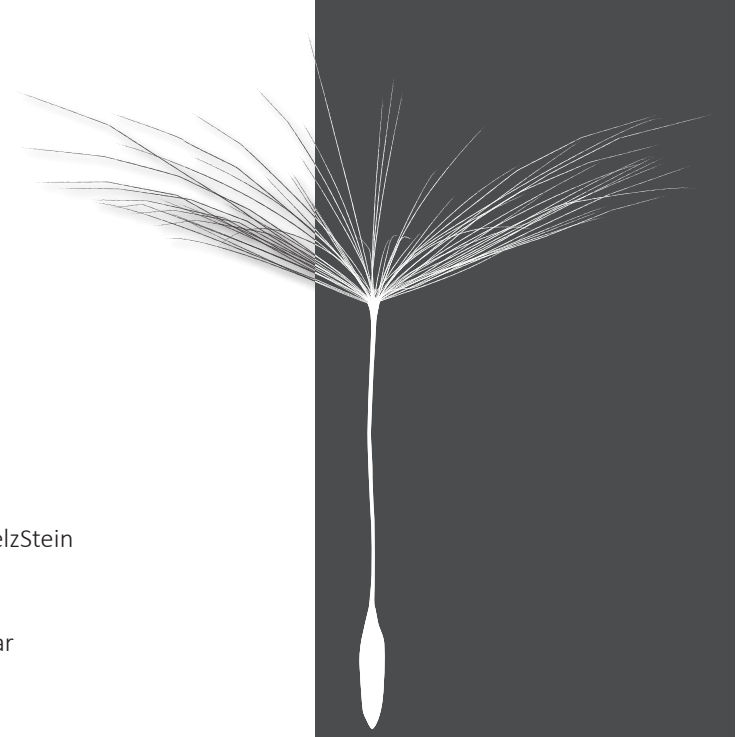
Developments in the treatment of advanced melanoma

Sloot, Sarah



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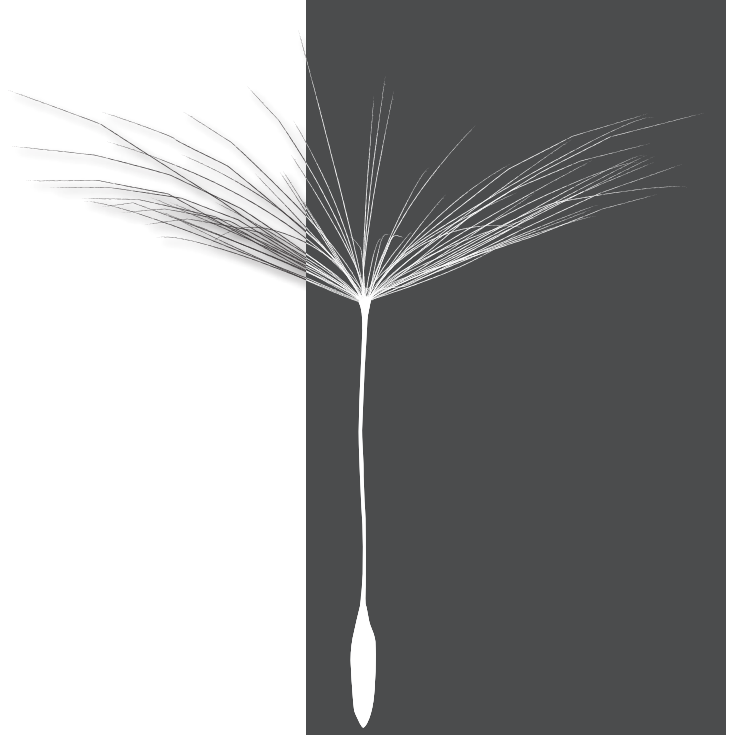

Paranymphs
Mijntje van Paridon
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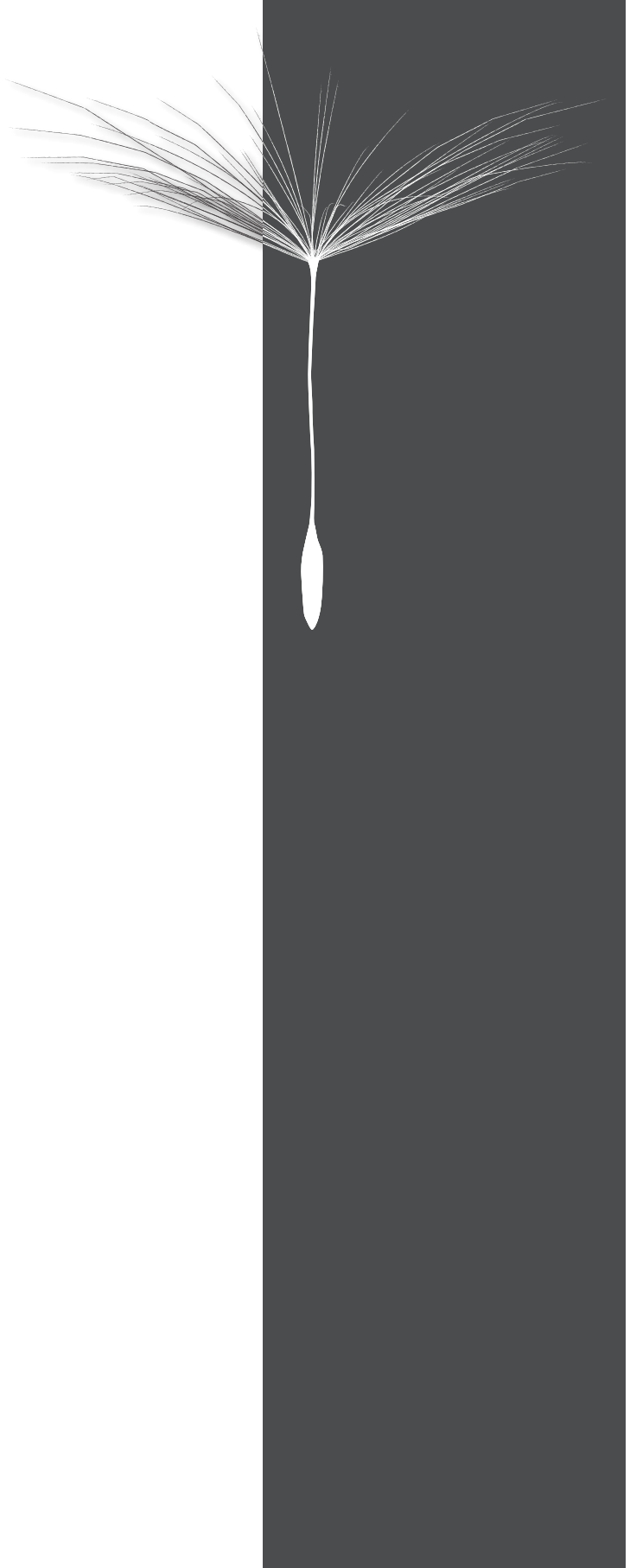


An introduction in melanoma

A brief history of cutaneous melanoma

Cancer has been described in humans as well as other vertebrates, preceding humankind itself.[1] In humans, cancer was first documented in the Edwin Smith papyrus.[2] Edwin Smith acquired this five meter long piece of papyrus in 1862 from an Egyptian merchant in antiquities in Luxor.[2,3] The papyrus was later established to be written in the 16th century B.C, being copied from an even older document written around 2500 B.C. It details the entire heritage of the famous Egyptian physician Imhotep, who lived around 2625 B.C. The document describes, amongst other diagnoses, fractures, suturing of wounds and cauterization with fire drills. It also includes a vivid description of what is to be believed the first documented cancer in men:

> *"If you examine [a case] having bulging masses on [the] breast and you find that they have spread over his breast; if you place your hand upon [the] breast [and] find them to be cool, there being no fever at all therein when your hand feels him; they have no granulations, contain no fluid, give rise to no liquid discharge, yet they feel protuberant to your touch, you should say concerning him: 'This is a case of bulging masses I have to contend with…bulging tumors of the breast mean the existence of swellings on the breast, large, spreading and hard; touching them is like touching a ball of wrappings, or they may be compared to the unripe hemat fruit, which is hard and cool to the touch"*[2]

Every case in the papyrus is followed by an extensive enumeration of therapies, but in this case, Imhotep falls silent. His brief conclusion states: *"There is none."*

Etymologically, melanoma stems from the word 'melanose' as introduced in 1804 by Rene Laennec, the inventor of the stethoscope.[1] Although the earliest physical evidence stems from the skeletons of pre-Colombian mummies (2400 years old), in whom diffuse melanotic metastases were found, cutaneous melanoma was not described as an entity until two millennia after the original description of cancer by Imhotep, when Hippocrates described 'fatal black tumors with metastases' in the 5th century B.C.[4] After these early observations, the medical profession grew silent on the topic for a long time. As with many diseases, surgeons were the physicians who made the first progress in treatment. The first reported surgical removal of melanoma was by the Scottish surgeon John Hunter 1787, although the tumor he

removed was not identified as a melanoma until 200 years later, when Bodenham reported that microscopic examination of the specimen confirmed that it was a melanoma.[1,5]

During the 19th century, large improvements were made in the documentation of melanoma based on thorough clinical observations. Despite numerous efforts and trials in treating the disease, patients with advanced melanoma had very poor prospects, as recognized early in Imhotep's papyrus. Until far into the 20th century, a bitter statement made by the French historian Voltaire rang true:

> *"Doctors are men who prescribe medicines of which they know little, to cure diseases of which they know less, in human beings of whom they know nothing."*

Prior to identifying any specific mutations as drivers in melanoma genesis and the role of the immune system in developing human cancer, the trial and error approach of cytotoxic agents utilized did little to improve the somber prognosis. Halfway through the 20th century William Norris identified that neither surgery nor medical treatments were effective once the melanoma was widely disseminated.[6] He advocated surgical excision, as well as ablation using caustic agents. William Norris is also credited with the first case series of melanoma, describing genetic, clinical and epidemiologic features of this disease in eight patients.[6] Herbert Lumley Snow published the first report on the rationale of a regional *elective* lymph node dissection (ELND) in 1892, postulating the 'anticipatory gland dissection' as a safe and easy procedure.[7,8] This procedure, where lymphadenectomy was performed in select patients as their surgeons saw fit, was later abandoned when four prospective trials showed no impact on survival and replaced by the sentinel lymph node biopsy.[9-12] This is different from a *therapeutic* lymph node dissection (TLND) for clinically apparent metastases, a *delayed* lymph node dissection (DLND) for metastases that become clinically apparent after the primary diagnosis, and *completion* lymph node dissection (CLND) which is done after a positive sentinel lymph node biopsy (SLNB).

Although wide local excision was used before, William Handley is widely considered as the father of this technique, describing the procedure in detail in 1907.[13] He advocated the removal of 5 cm of subcutaneous tissue down to the level of muscle fascia along with radical removal of lymph nodes. His adage was upheld for nearly 50 years. In the 1960's, Olsen and Wong published studies that established the 5 cm excision margin as a safe and effective procedure.[14,15] Handley's wide excisions were later abandoned after a series of randomized trials supported the safety of narrower margins.[16-24] Current consensus in the majority of Western countries is to excise melanoma *in situ* with 5 mm margins, melanoma ≤1 mm thick with 1 cm margins, 1.01-2 mm with 1-2 cm margins and >2 mm with 2 cm margins.[25-27] Since these resections margins are under debate, the MELMART trial was initiated in 2014 (NCT02385214). As smaller resection margins are expected to improve quality of life patients in this trial are randomized between a 1 cm and 2 cm excision margin and assessed for rate of local recurrence and melanoma specific survival.[28]

Although rare, spontaneous regression has been reported in melanoma. The reported incidence in primary melanoma ranges from 4-15%, however, regression of melanoma metastases is uncommon and reported in less than 1% of patients.[29]

Knowledge about and treatment options for melanoma were advancing slowly but surely, but during the 20th century surgeons became increasingly disappointed with the morbidity of lymph node dissections. Data became available that only 20% of all patients with melanoma harbored lymph node metastases. Randomized trials comparing comparing ELND to DLND failed to show a survival benefit.[9,10,30]

It was during this era that locoregional and systemic therapies started to emerge. In 1958, Creech and Krementz published on isolated limb *perfusion* for in-transit disease, where the blood circulation of a limb is temporarily isolated using a tourniquet.[31] This allows for higher chemotherapy concentrations than can be systemically administered. This method was refined in the early 1990's, when isolated limb *infusion* was invented, which eliminates the extensive surgical procedure.[32] These procedures have dramatically decreased the need for limb amputations. Chapter 4 will provide more detail on the development and success rates of these therapies.

The year 1962 saw the dawn of systemic therapies when a case series was published treating patients with melphalan, an alkylating chemotherapeutic agent, and autologous bone marrow transplants. Though melphalan showed some efficacy, duration of action was short and its use was limited by severe toxicity.[1] In 1967 the FDA approved its first systemic therapy, hydroxyurea, for the treatment of metastatic melanoma.[33] Hydroxyurea is a cytotoxic agent that acts as a ribonucleotide reductase inhibitor, thereby inhibiting DNA synthesis. Its efficacy is very limited[34,35]. Dacarbazine, an alkylating agent, was approved in 1976 and remained the standard therapy for decades.[33]

After the approval of hydroxyurea and dacarbazine, it took nearly 30 years for more drugs to come to market: interferon-α, a cytokine that enhances HLA antigen presentation, activates natural killer (NK) cells and also has a direct inhibiting effect on melanoma cells, was approved in 1996 and interleukin-2 (IL-2), an immuno-stimulatory cytokine mainly involved in T cell proliferation, was approved in 1998.[33] Another decade passed before developments truly sped up. For the first time multiple promising agents for the treatment of metastasized melanoma progressed through trials simultaneously.

Since 2011, the US Food and Drug Administration (FDA) and the Europees Geneesmiddelen Agentschap (EMA) have approved nine different agents. That year was a landmark with the introduction of ipilimumab, an anti-CTLA 4 antibody, and vemurafenib, a BRAF inhibitor.[36,37] In 2013 a second BRAF inhibitor, dabrafenib, was approved.[38] Other approved drugs are the MEK inhibitors trametinib and cobimetinib, the anti-PD1 antibodies nivolumab and pembrolizumab and the intralesional oncolytic virus talimogene laherparepvec.[33,39-43]

As melanoma is relatively insensitive to radiation therapy, it is rarely used for treatment. Newer approaches are being developed, such as adjuvant strategies.[44,45] Adjuvant radiotherapy in patients undergoing a lymphadenectomy for a palpable lymph node field relapse decreased local relapse to 21%, as compared to 36% for observation alone, after six years of follow-up, but did not decrease overall relapse rate.[46-48] In cutaneous melanoma, radiotherapy is therefore used to increase locoregional control after therapeutic lymphadenectomy and also to treat bone and brain metastases.

A more comprehensive overview of locoregional and systemic options will be provided in the paragraph 'Treatment' (page 25), after reviewing epidemiology, clinical presentation, staging, prognosis and pathology.

Epidemiology

In 2017, in the United States an estimated 87,110 people will develop melanoma and 9,730 people are expected to succumb to the disease in a population of 326 million people.[49] For the Netherlands, the most recent available numbers are from 2016. In that year 6,787 people were diagnosed with melanoma of the skin and external genitalia in a population of 17 million people.[50] More than a tenfold of that number are classified as living with the disease, which is largely driven by the excellent prognosis (>92% 5-year survival for melanomas ≤1 mm in depth) if melanoma is diagnosed at an early stage. Approximately 78% of all newly diagnosed patients present with stage I melanoma (paragraph 'Staging', page 17).[51] Its incidence renders melanoma the 6th most common cancer, representing 4.5% of all new cancer cases in the US.[52] The median age at diagnosis is 57 years and the median age at death is 67 years.[44,53]

The lifetime risk of melanoma of the skin is 1 in 50 and still on the increase, though the rise in incidence has slowed down.[50,52] In comparison, incidence was only 1 in 1500 in 1935 and 1 in 250 in 1980.[44] Diagnoses of melanoma in situ are rising faster than other types of melanoma. The increase in incidence is therefore multifactorial and should be contributed to a combination of better screening and earlier detection and an increase in exposure to ultraviolet light which has long been known to be a risk factor for melanoma and nonmelanoma skin cancers.[54] The popularity of tanning beds contributes to this increased exposure. Contrary to other skin cancers, which are mainly associated with cumulative sun damage, intermittent high sun exposure, as indirectly assessed by taking a history of sunburns, has been linked to melanoma. Sunburns in childhood carry the highest risk.[44]

UVA, UVB and ultraviolet (UVR) solar radiation penetrate into the skin and cause DNA damage. The mechanism by which DNA damage occurs is complex, but a simplified explanation would be that UVR leads to single-strand breaks, double-strand breaks and cross-links between DNA strands; both UVA and UVB induce the synthesis of pyrimidine dimers; UVA in addition contributes through the formation of free radicals; and UVB also leads to the formation of 6,4 photoproducts, a specific link between two pyrimidine rings.[54] Two of four base pairs used as a building block in DNA, i.e. cytosine and thymine, are pyrimidine derivatives. Formation of dimers interferes with base pairing during DNA replication and thereby leads to mutations. Free radicals, single-strand breaks and double-strand breaks lead to immediate DNA damage upon replication.[54]

A small percentage of melanomas are discovered in a disseminated phase without a primary melanoma being identifiable. A large case series showed an incidence of 2.6% for these so-called melanomas of unknown primary.[55] Based on mutational analysis, melanomas of unknown primary have a mutation profile consistent with cutaneous sun exposed melanomas and may arise in lymph nodes or as distant metastasis from cells that have migrated from the skin.[43,44] Survival is similar to melanoma with macroscopic lymph node metastasis.[55] When it comes to treatment decisions, these melanomas should therefore be regarded as cutaneous melanoma.[56,57]

Men are more susceptible to developing melanoma than women (relative risk (RR) 1.74), with men developing melanoma most commonly on the trunk and women on the extremities.[54,58] This is hypothesized to be caused by patterns in behavioral sun exposure, as recent years have seen an increase in trunk melanomas in women.[54,59,60] Other risk factors include age (RR 1.02 per year increase), family history (RR 2.19 for positive family history), number of naevi (RR 3.08 with 10+ >3 mm naevi on extremities), hair color (as compared to light brown, RR 0.60 for black hair, RR 1.21 for blonde (non-significant) and 2.05 for red hair) and history of sunburns (RR 2.36 for >10 severe sunburns).[58] Smoking is associated with an increase in Breslow thickness (0.25 mm), ulceration and positive SLNB.[61]

Of all melanomas, 5-12% are estimated to be hereditary. The most common driver gene in this group of patients is cyclin-dependent kinase inhibitor 2A (CDKN2A), which codes for proteins acting as tumor suppressors in the cell cycle.[62] Autosomal dominant inheritance of germline *CDKN2A* mutations has been implicated in approximately 20-40% of familial melanoma; the mutation frequency varies between different geographical regions.[62]

Other, more sporadic, entities are ocular melanoma, acral melanoma and mucosal melanoma, which carry different characteristics and a different prognosis. These entities fall outside the scope of this introduction.

Clinical presentation

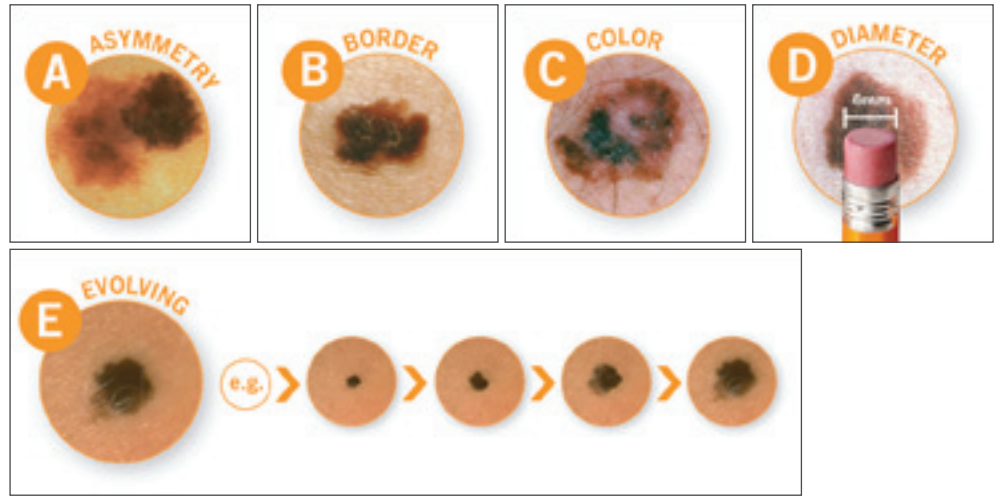
The skin is the largest organ of the body and consists of the epidermis and dermis. Melanoma originates in melanocytes, found in the epidermis, which makes the tumor distinctly different from both carcinomas - originating in epithelial tissues - and sarcomas - arising in mesenchymal tissues. Approximately 25% of melanomas originate in pre-existing lesions, so the majority of tumors arise *de novo* with no identifiable precursor lesion. A *de novo* melanoma arises when an oncogenic stimulus, often excess sunlight, as described above, acts on these melanocytes, leading to proliferation of the mutated melanocytes.[63]

Clinically, the lesion progresses from a light tan macule, to a small pigmented macule without significant aplasia, to a lesion manifesting noticeable abnormalities as the mutated melanocytes spread throughout the epidermis.[63] The tumor is usually dark in origin, reflecting the presence of melanin in the upper levels of the epidermis. Between 1.8 and 8.1% of melanomas is amelanotic, lacking the characteristic dark color.[64] Metastasis occurs when the tumor starts extending to the dermis and subcutaneous fat, where it can invade blood vessels and lymphatics. This is also the stage at which ulceration, nodule formation and regression may occur.[63]

Diagnosing melanoma starts with a skin exam, sometimes aided by the use of a dermatoscope, a handheld device specifically designed to examine the skin using skin surface miscroscopy.[65] Since the different clinical presentations of melanoma only constitute a few diagnoses out of the more than 1500 skin conditions that have been described, skin assessment is more accurate in the hands of an experienced clinician, leading to a lower number of false negative excised lesions.[65] The number of melanocytic lesions that have to be excised to diagnose one melanoma, otherwise known as number needed to treat, varies highly depending on the experience of the assessing clinician, from 20-40 for general practitioners at nonspecialized clinics, to 19-28 for general practitioners at skin cancer clinics, to 4-8 for dermatologists at specialized clinics.[65]

The risk of a mole being melanoma can be assessed using the ABCD(E) rule, which encourages a systematic evaluation of the lesion in search for signs of asymmetry, border irregularity, color variation, diameter >6 mm and evolution (Figure 1).[66] It is important to combine these factors as the sensitivity and specificity of the individual criteria are limited; sensitivities of 57%, 57%, 65%, 90%, and 84% and specificities of 72%, 71%, 59%, 63%, and 90% for the five traits have been reported, respectively.[66,67] If a lesion is suspect for a melanoma, a biopsy will be done to obtain a histological diagnosis. Incisional as well as excisional biopsies may be done, however, excisional biopsy is preferred and recommended by the AJCC as it encompasses the entire lesion and provides the best information for pathological diagnosis and staging.[51] The biopsy must achieve adequate depth and circumference, to facilitate complete tumor removal, and can be done using narrow margins. Microscopically, atypical melanocytes will be visible throughout the various layers of the skin, depending on the stage of progression and Breslow depth.[63] Upon histological/pathological confirmation of a melanoma diagnosis

Figure 1 - ABCDE rule in melanoma[66]



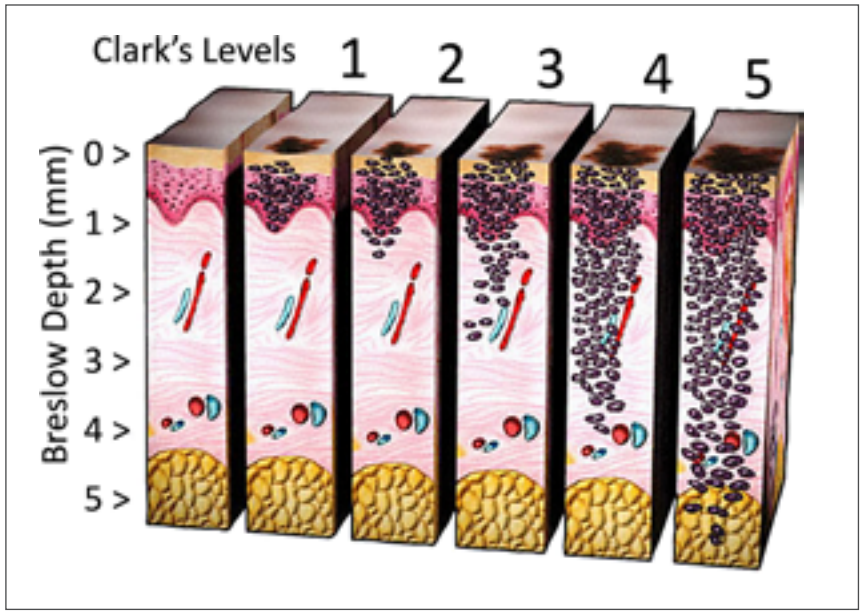
and Breslow depth, re-excision with wider margins should be performed if necessary as described above.[25-27] Incisional biopsy of the most atypical part of the lesion can be an option for large lesions, lesions that have a low suspicion for melanoma, or are located on face or acra.[68] This type of biopsy has the risk of underestimating the true depth of a lesion as it samples only part of a lesion.

For high risk patients as identified by pathology results and clinical symptoms/exam findings, evaluation for disseminated disease is performed using imaging techniques, e.g. FDG-PET or CT-scans.[69,70] Melanoma can metastasize to almost all organs, but the most frequently involved sites are liver, bones, lungs and brain. For the brain, MRI is the modality of choice. Due to inability to identify micrometastases and the low probability of metastasis in early stage disease, these imaging techniques have a limited role in stage I and II tumors.[71,72]

Staging

The first effort to use a staging system for melanoma was introduced by Wallace Clark in 1969, subdividing melanoma into five anatomic levels of invasion.[73] Clark's levels use the level of downward invasion of the melanoma. Five-year survival ranges from over 99% for Clark level I (melanoma in situ) to 55% for Clark level V. Clark concluded that ELND should be restricted to patients with level III, IV and V lesions.[7] In 1970 Alexander Breslow introduced Breslow thickness as a measurement, which is still in use today (Figure 2).[74] Breslow thickness

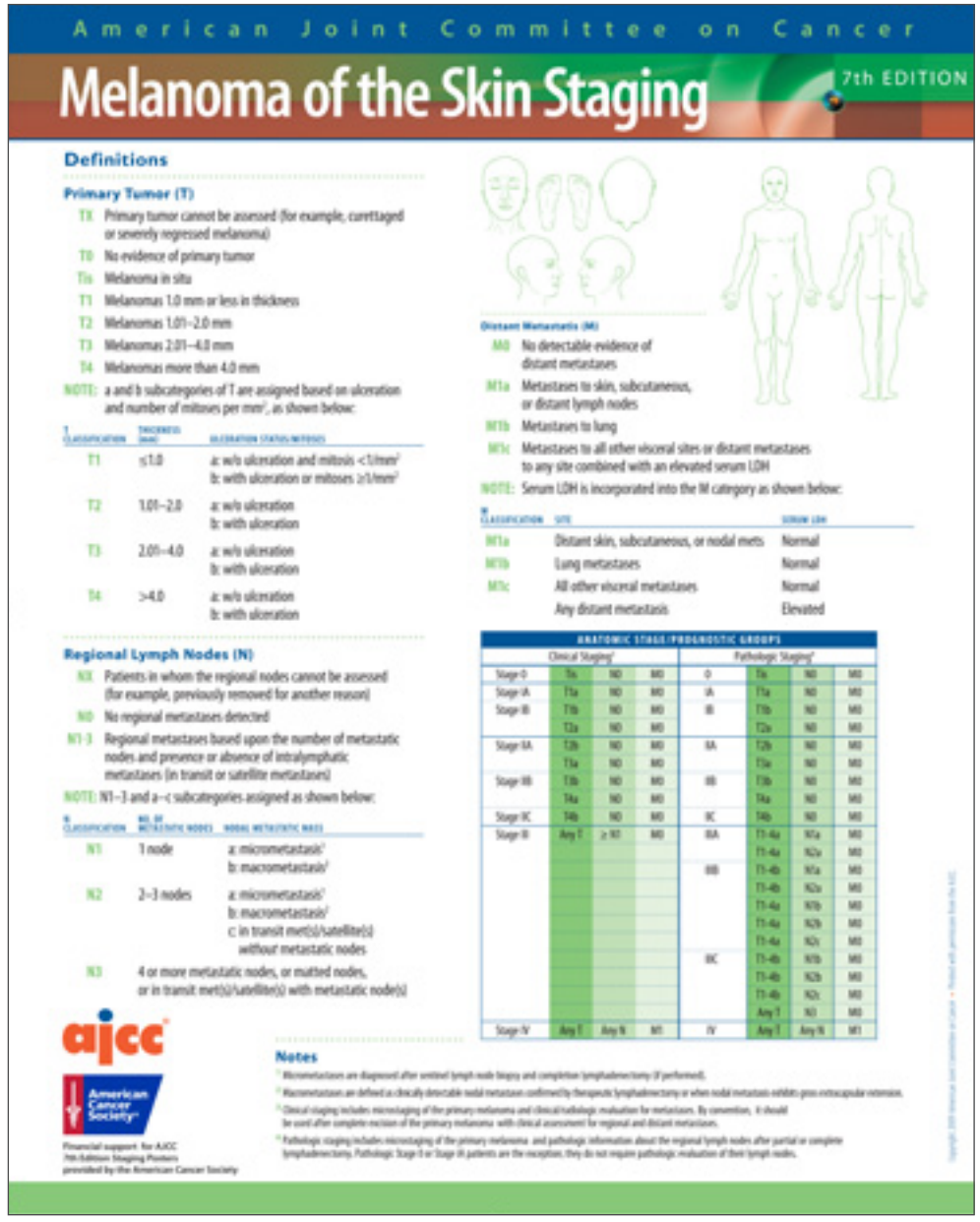
Figure 2 - Differences and overlap between Clark levels and Breslow depth[75]



is a quantitative measurement of the total vertical depth of a melanoma, as measured in millimeters, and has become a major prognostic factor for localized melanoma. It is also used to identify patients who benefit from sentinel lymph node biopsy after surgery.

The AJCC introduced a more precise staging system in 1998 and has updated this system since. The 7th edition of the melanoma staging system was published in 2009. The 8th edition will be implemented at the start of 2018.[51] The AJCC staging system uses three parameters, local advancement (T), a combination of Breslow depth and ulceration, lymph node status (N) and distant metastasis (M).[51] It is therefore also referred to as the TNM staging system. Regional metastasis is defined as metastasis to the regional lymph nodes, the location of which depends on the location of the primary melanoma, or in-transit/satellite metastasis, which is metastasis in between the primary melanoma and the regional lymph node station. Distant metastasis is all other skin and lymph node metastasis and also includes visceral metastasis. The three parameters (T, N and M) are then combined into four categories (I-IV, Figure 3). Stage I represents limited local disease, stage II locally advanced disease, stage III regionally advanced disease and stage IV distant metastasis. The AJCC staging system, and before that the Clark and Breslow systems, have guided patient selection in many trials.

Figure 3 - AJCC staging system for melanoma[51]


American Joint Committee on Cancer

Melanoma of the Skin Staging 7th EDITION

Definitions

Primary Tumor (T)

TX Primary tumor cannot be assessed (for example, curettaged or severely regressed melanoma)
T0 No evidence of primary tumor
Tis Melanoma in situ
T1 Melanomas 1.0 mm or less in thickness
T2 Melanomas 1.01–2.0 mm
T3 Melanomas 2.01–4.0 mm
T4 Melanomas more than 4.0 mm
NOTE: a and b subcategories of T are assigned based on ulceration and number of mitoses per mm², as shown below:

T Classification	Thickness (mm)	Ulceration Status/Mitoses
T1	≤1.0	a: w/o ulceration and mitosis <1/mm² b: with ulceration or mitoses ≥1/mm²
T2	1.01–2.0	a: w/o ulceration b: with ulceration
T3	2.01–4.0	a: w/o ulceration b: with ulceration
T4	>4.0	a: w/o ulceration b: with ulceration

Regional Lymph Nodes (N)

NX Patients in whom the regional nodes cannot be assessed (for example, previously removed for another reason)
N0 No regional metastases detected
N1-3 Regional metastases based upon the number of metastatic nodes and presence or absence of intralymphatic metastases (in transit or satellite metastases)
NOTE: N1–3 and a–c subcategories assigned as shown below:

N Classification	No. of Metastatic Nodes	Nodal Metastatic Mass
N1	1 node	a: micrometastasis[1] b: macrometastasis[2]
N2	2–3 nodes	a: micrometastasis[1] b: macrometastasis[2] c: in transit met(s)/satellite(s) without metastatic nodes
N3	4 or more metastatic nodes, or matted nodes, or in transit met(s)/satellite(s) with metastatic node(s)	

Distant Metastasis (M)

M0 No detectable evidence of distant metastases
M1a Metastases to skin, subcutaneous, or distant lymph nodes
M1b Metastases to lung
M1c Metastases to all other visceral sites or distant metastases to any site combined with an elevated serum LDH
NOTE: Serum LDH is incorporated into the M category as shown below:

M Classification	Site	Serum LDH
M1a	Distant skin, subcutaneous, or nodal mets	Normal
M1b	Lung metastases	Normal
M1c	All other visceral metastases	Normal
	Any distant metastasis	Elevated

Anatomic Stage/Prognostic Groups	Clinical Staging[3]			Pathologic Staging[4]			
Stage 0	Tis	N0	M0	0	Tis	N0	M0
Stage IA	T1a	N0	M0	IA	T1a	N0	M0
Stage IB	T1b	N0	M0	IB	T1b	N0	M0
	T2a	N0	M0		T2a	N0	M0
Stage IIA	T2b	N0	M0	IIA	T2b	N0	M0
	T3a	N0	M0		T3a	N0	M0
Stage IIB	T3b	N0	M0	IIB	T3b	N0	M0
	T4a	N0	M0		T4a	N0	M0
Stage IIC	T4b	N0	M0	IIC	T4b	N0	M0
Stage III	Any T	≥ N1	M0	IIIA	T1–4a	N1a	M0
					T1–4a	N2a	M0
				IIIB	T1–4b	N1a	M0
					T1–4b	N2a	M0
					T1–4a	N1b	M0
					T1–4a	N2b	M0
					T1–4a	N2c	M0
				IIIC	T1–4b	N1b	M0
					T1–4b	N2b	M0
					T1–4b	N2c	M0
					Any T	N3	M0
Stage IV	Any T	Any N	M1	IV	Any T	Any N	M1

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Notes

[1] Micrometastases are diagnosed after sentinel lymph node biopsy and completion lymphadenectomy (if performed).
[2] Macrometastases are defined as clinically detectable nodal metastases confirmed by therapeutic lymphadenectomy or when nodal metastasis exhibits gross extracapsular extension.
[3] Clinical staging includes microstaging of the primary melanoma and clinical/radiologic evaluation for metastases. By convention, it should be used after complete excision of the primary melanoma with clinical assessment for regional and distant metastases.
[4] Pathologic staging includes microstaging of the primary melanoma and pathologic information about the regional lymph nodes after partial or complete lymphadenectomy. Pathologic Stage 0 or Stage IA patients are the exception; they do not require pathologic evaluation of their lymph nodes.

Prognosis

Melanoma has an excellent prognosis when discovered at an early stage, however, overall survival (OS) drops sharply as stage progresses.[51] From a cellular perspective, metastases are inefficient and uncommon. Only 0.01%-0.03% of cells in a primary tumor metastasize to other organs, offering an explanation for the relatively low rate of tumors that metastasize.[76] Although melanoma is well known for its capability of late metastasis, the vast majority of metastases happen early in the disease.

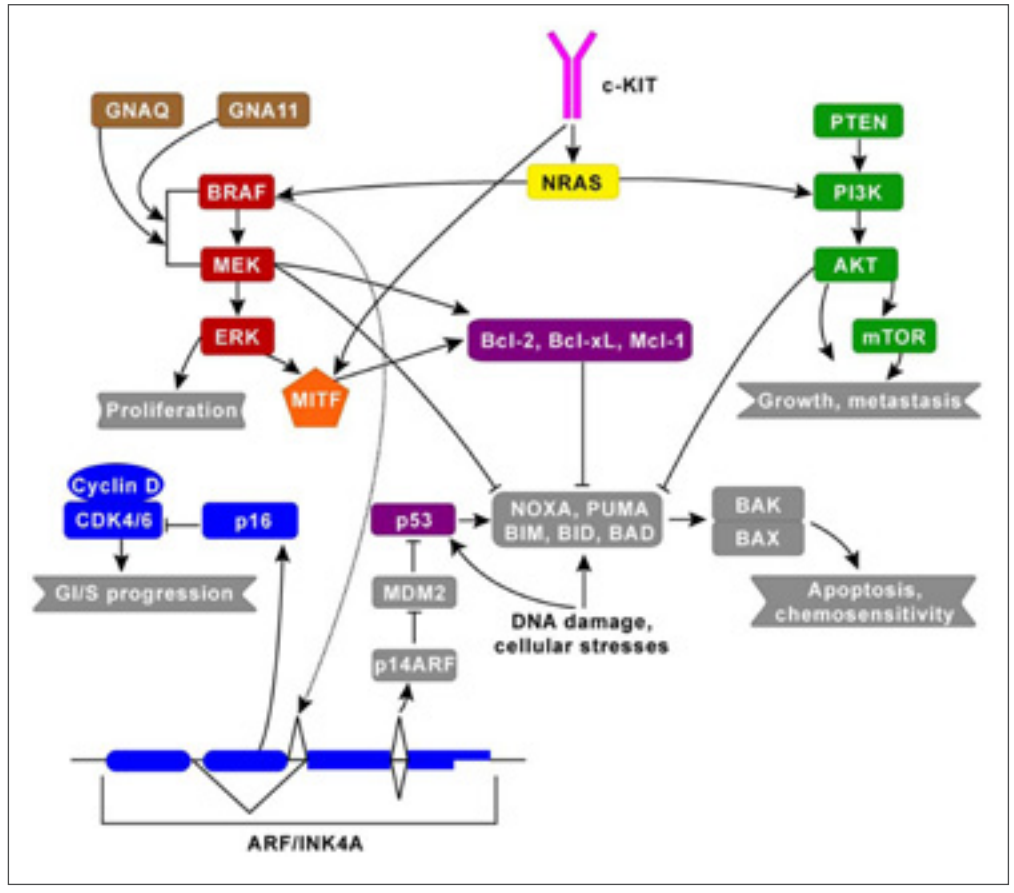
For stage IA, IB, IIA, IIB, and IIC 5-year survival rates are 97%, 92%, 81%, 70% and 53%, respectively. Five-year survival for locoregional metastasis is 78% (stage IIIA), 59% (stage IIIB) and 40% (stage IIIC).[51] Once melanoma has metastasized distantly, 5-year survival drops to 15-20%, although these rates are expected to improve upon the recent introduction of BRAF-targeted drugs, checkpoint inhibitors and combination therapies.[26,36,37,40,77-79] Greater Breslow thickness, tumor location, gender, tumor ulceration, microsatellites, mitotic rate, regression, sentinel lymph node, number of tumor-infiltrating lymphocytes and angiolymphatic invasion have all been associated with prognosis.[21,44] Out of these, long-term follow-up reveals the strongest predictors for recurrence are Breslow depth (as compared to Breslow depth ≤1 mm, odds ratios (OR) for ≥1-2 mm, >2-4 mm and >4 mm are 2.84, 6.08 and 8.81, respectively), ulceration (OR 2.41 for ulcerated tumors vs. non-ulcerated tumors) and sentinel lymph node status (2.74 for positive SLNB vs. negative SLNB).[80,81] Once metastasis has occurred, the site of first metastasis is the most important prognostic factor (hazard ratio (HR) 1.3 for metastasis to the regional lymph nodes vs. satellite/in-transit recurrence, and HR 5.5 for distant metastasis vs. satellite/in-transit recurrence, $p < 0.001$).[82]

Pathology, molecular biology and actionable targets

After biopsy the diagnosis of a skin lesion depends on pathologic assessment. The Edwin Smith Papyrus is the earliest source of pathological anatomy, however, a pivotal development in current tissue diagnosis was the invention of the microscope, which changed concepts of disease from whole organs to separate cells and enabled cytological and histological assessment of tumors.[83] Paraffin embedding was added to the repertoire by Edwin Klebs in 1869.[83] Cytological characteristics of melanoma include the presence of atypical and necrotic melanocytes and melanocytes undergoing mitosis.[84] Histologically, melanoma is classified based on asymmetry, poor circumscription and the presence of irregularly distributed melanocytes occupying the epidermis, dermis and adjacent tissues. Melanin is irregularly distributed in the lesion.[63] Chemical and immunohistochemical stains may aid the diagnosis. Various markers are used, such as S-100B, HMB45, Mart-1, tyrosinase, MITF, NKI/C3, CD10 antigen and vimentin.[71,85] To optimize sensitivity and specificity a combination of these diagnostic aids should be used.

Recent years have seen the emergence of molecular biology, which has the potential to identify gene mutations that can be used as therapeutic targets. In 2010, a melanoma cell line was the first cell line being reported in a whole genome sequencing study cataloguing the somatic mutations in cancer.[86] Step by step this has led to a better understanding of cellular pathways and possible actionable targets. Figure 4 shows a simplified representation of eight pathways identified in melanoma. Our understanding of the molecular background of cancer has advanced since the publication of this model, but it still serves as a good base for pathways found in melanoma. Abnormalities in the MAPK pathway, also known as Ras-Raf-MEK-ERK pathway, are found in 80% of patients with melanoma.[86,87]

Figure 4 - The Melanoma Molecular Disease Model[87]



Although NRAS mutations were the first oncogenes described in melanoma in 1984, the development of targeted therapy in metastatic melanoma truly has its footing with the identification of activating mutations in BRAF, a serine threonine kinase, in 2002 by Davies et al.[88] BRAF was first identified in 1988 as the transforming gene in a sample of Ewing's sarcoma.[89] Mutations in exon 15 of the BRAF gene are now known to occur in 40-60% of cutaneous melanomas, with the most common being the V600E mutation, an amino acid substitution at position 600 in BRAF from a valine to a glutamic acid, in >85% of these patients.[90] This gain of function change leads to constitutive activation of the MAPK pathway, resulting in cell growth, proliferation and increased invasive potential. BRAF is a promising target for systemic treatments as these tumors are generally dependent on a single oncogene, like Bcr-Abl in CML and c-KIT in gastrointestinal stromal tumors. While BRAF mutations alone are not able to transform melanocytes into malignant melanoma cells, they create a more aggressive phenotype of melanoma and prior to the development of targeted agents patients with metastatic melanoma harboring BRAF mutations had worse overall survival than the average population.[91] Curiously enough, BRAF is not exclusively found in melanomas, but is also present in the majority of benign naevi. Most of these naevi are in a state of permanent growth arrest (senescence) following the acquisition of mutant BRAF, which explains why so few naevi progress to melanoma.[92]

The molecular disease model also demonstrates the benefit of combination therapy. By e.g. combining BRAF inhibitors and MEK inhibitors, the MAPK pathway is inhibited on two separate levels. This increases response rates and delays the onset of resistance, as further discussed in the paragraph 'Epidemiology' (page 14).

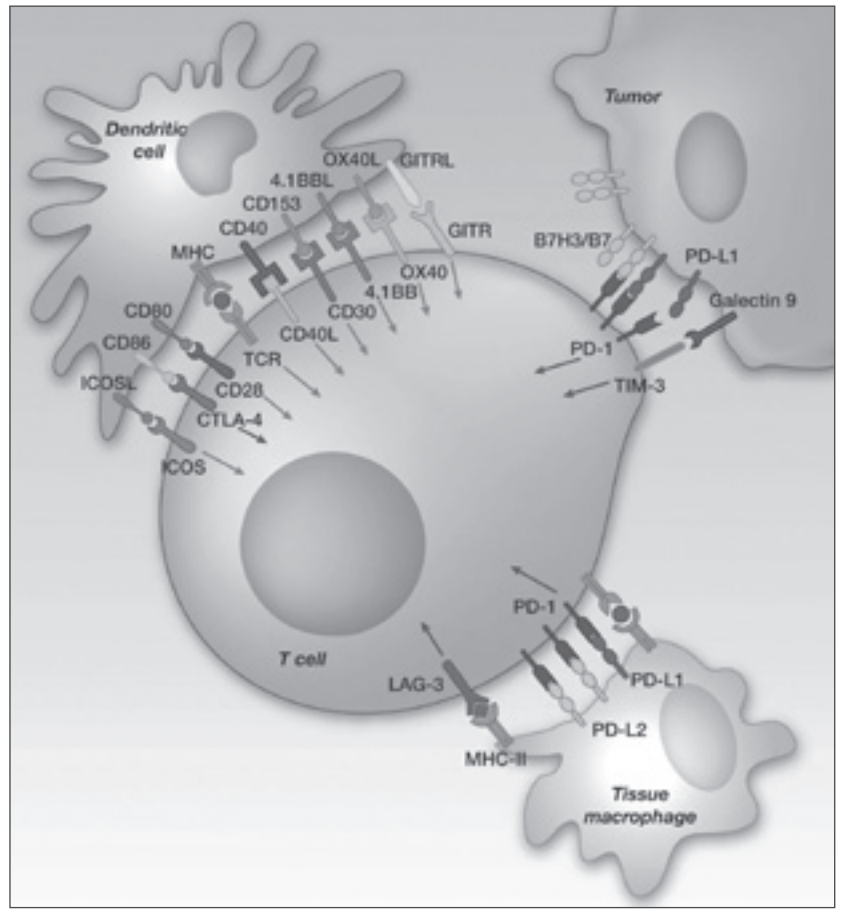
Another topic of interest in tumor biology is biomarkers, as these would be able to predict prognosis and, in an ideal situation, will assist in selecting patients for systemic treatment based on anticipation of response, thereby tailoring oncologic treatment to the patient. Lactate dehydrogenase (LDH) and S-100B have been found to serve as biomarkers and may be used to measure response to therapy. Increased levels in peripheral blood are associated with worse outcome.[71] LDH, which is included in the AJCC staging system, can be very useful for detecting distant metastasis; however, it is also associated with other malignancies and has low sensitivity.[51] S-100B is located in the cytoplasm of melanoma cells and its presence in peripheral blood is thought to be caused by loss of integrity of these cells. It is strongly correlated with both tumor burden and survival.[71] In a recent small patient series, preoperatively measured S-100B was the strongest predictor for non-sentinel node positivity in patients planned for CLND.[93]

Other implied biomarkers are PD-1 and PD-L1 expression on the tumor, which have been used in phase 3 studies of agents directed against these proteins. PD-L1 expression did have an effect on overall response rate to PD-L1 inhibitors in a review (45% for PD-L1 positive patients vs. 27% in PD-L1 negative patients), a pattern that can be seen in the treatment of other tumor types as well.[94] As responses are still seen in the PD-L1 negative group, this marker is not yet ready to be used to select patients. As of yet, these biomarkers have not resulted in a better selection of patients or seen a translation into survival benefit and their use is mainly prognostic, however, as more data become available utility of biomarkers may expand.

Melanoma and the immune system

Tumors may evolve using a process called immunoediting, which consists of three phases: elimination, equilibrium and escape.[95] Through the process of immunosurveillance (elimination), the immune system continuously checks cells to distinguish invading cells, such as bacteria and viruses, from the body's own and tumor cells from their non-cancerous counterparts.

Figure 5 - Costimulatory and coinhibitory ligand-receptor interactions between a T cell, dendritic cell, tumor cell and macrophage[96]



To do this, it uses checkpoints; molecules on immune cells that need to be activated or inactivated to start an immune response.[96] These checkpoints use the proteins that are expressed on the cellular surface or foreign cells to recognize and eliminate threats. The phrase 'immune checkpoints' thus refers to a plethora of pathways in the immune system that regulate immune responses (Figure 5). Immune tolerance and evasion by tumor cell populations can be achieved through these immune checkpoints. Cytokines can have similar actions, but typically have diverse functions on immune cell populations, including immune suppression (e.g. IL-6 and IL-8) and stimulation of T cell activity and proliferation (IL-2).[97,98] Both cells in the immune system and tumor cells express immune checkpoint molecules, which can have either stimulatory or inhibitory effects on immune cells.[99] E.g. PD-1 on physiological cells acts as an off-switch to keep the immune system from attacking the own body. When tumor cells express PD-L1, one of the ligands of the PD1 receptor, the binding of these two proteins leads to the immune system not reacting to the tumor cell.
The tumor cells surviving the elimination phase enter into equilibrium between immunologic pressure and tumor growth. It is thought that this process may last up to many years, before the tumor acquires insensitivity to immunologic detection and/or elimination through genetic or epigenetic changes, escapes the equilibrium and starts to grow uncontrollably.[95]

T cells require three signals for optimal T cell recognition and generation of an adaptive T cell immune response: first, recognizing antigen presented by major histocompatibility complexes (MHCs); then, a signal activating T cells, e.g. interaction of the CD28 costimulatory marker on T cells with CD80/CD86 on antigen-presenting cells. 4-1BB and OX40 can also initiate this step. Third, amplification of T cell receptor signaling and secretion of cytokines (e.g. IL-2), which then differentiate and activate T cells. T cell activation leads to release of cytotoxic granules (e.g. granzyme and perforin) and direct induction of apoptosis (e.g. Fas-Fas ligand interactions), leading to tumor cell death.[100]

Melanoma is known to be a tumor with a high mutational load and should thus be recognized by the immune system as foreign. The fact that the tumor is able to evade the immune system opens up treatment possibilities in priming the immune system. Cytokine therapies such as IL-2 and interferon-α have served as proof of principle that the immune system can be stimulated to produce antitumor responses against melanoma and other tumor types. While IL-2 has the potential to produce durable responses in patients with advanced melanoma, objective responses occur in less than 20% of patients and serious toxicities are observed in most patients.[97] The search for more effective and tolerable immunotherapies was rewarded with the development of CTLA-4 and PD1/PD-L1 antibodies.[101]

The immunoglobulin CTLA-4 (CD152) is a transmembrane receptor exclusively expressed on T cells. CTLA-4 competes with the costimulatory CD28 molecule for binding CD80/CD86, leading to downregulation of T cell receptor signaling.[99,100,102] CTLA-4 knockout mice experience lethal systemic immune hyperactivation, thereby demonstrating the importance of the immunoglobulin.[103]
In response to the CD28-CD80/CD86 interaction, tumor cells can develop resistance by aberrantly overexpressing inhibitory ligands (e.g. PD-L1) that downregulate T cell effector function through T cell exhaustion or anergy.[102] The binding of ligand PD-L1 on antigen presenting cells and tumor cells to PD1 on T cells delivers an inhibitory signal to the T cell, inhibiting T cell receptor signaling, activation of IL-2 production and T cell proliferation.[99] Under normal physiological circumstances, the main role for PD1 is to limit effector T cell responses in peripheral tissues at the time of an inflammatory response to infection and to limit autoimmunity, thereby protecting against immune-mediated tissue damage.[100,101] PD1 is a transmembrane protein expressed on activated T cells, B cells and monocytes. Binding of anti-PD-L1 to PD1 downregulates T cell activation, so blocking either PD1 or PD-L1 interrupts this signal, leading to activation of T cells and removing the inhibitory signal that keeps the immune system from attacking the tumor.[100] The paragraph 'Epidemiology'(page 14) will provide more details on clinical efficacy of these agents.
The other ligand that has been described for PD1 is PD-L2. PD-L2 is not as widely expressed as PD-L1 and its role in cancer genesis is less clear. While studies have found that patients with tumors expressing PD-L1 have an impaired survival, no such correlation has been found for PD-L2. It is too early to say whether PD-L2 is a viable target.[104] Antibodies against PD-L2 are being developed, as are antibodies against some of the other receptors shown in Figure 5, such as OX40 and 4-1BB.

Treatment

A broad range of therapies have been used over the years to treat melanoma, however, Imhotep's somber treatment statement - *'There is none'* - rang true until far into the 20th century. Surgery is still the mainstay of melanoma treatment (paragraph 'Epidemiology', page 14). For patients with isolated in-transit metastasis (i.e. no other identified metastases) in the extremities and liver metastasis, locoregional perfusion/infusion techniques or intralesional injection may be used (paragraph 'Epidemiology', page 14).[105-107] As mentioned in the paragraph 'A brief history in cutaneous melanoma' (page 11), the 2000's have seen the dawn of new checkpoint blockade and BRAF-targeted systemic therapies. This will be further discussed in the paragraph 'Epidemiology' (page 14).

Surgical treatment

Surgery is a cornerstone in melanoma treatment. As described in the paragraph 'A brief history in cutaneous melanoma' (page 11), for stage I-II patients, surgical excision with 5 mm margins for melanoma in situ is advocated, 1 cm margins for melanomas ≤1 mm, 1-2 cm margins for melanoma 1.01-2 mm and 2 cm margins for melanomas >2 mm thick, combined with a sentinel node biopsy in melanomas ≥1 mm in depth.[25-27,51] For select stage III-IV patients, surgery provides an improved chance at long term survival, therefore metastasectomy should be considered in patients with limited metastasis.[108-110] Most locoregional disease will

be amenable to resection aiming to render the patient free of disease.[111,112] However, some patients will present with unresectable bulky adenopathy due to surgical limitations such as involvement of neurovascular structures. Similarly, as many as 24% of patients with recurrent locoregional melanoma have satellite and/or in-transit disease not amenable to complete resection.[82]
The newest development in surgical treatment is to perform robotic-assisted or videoscopic surgeries.[113,114] This has mainly been described for inguinal lymphadenectomies and allows for less invasive procedures while maintaining an adequate lymph node yield. Shorter hospital stays and less wound complications have been described in a small number of patient reports, however, more extensive data are needed to establish the role of these procedures in melanoma treatment.[113,114]

Sentinel lymph node biopsy

The sentinel lymph node biopsy (SLNB) is an intraoperative procedure where the status of the draining lymph node basin is assessed by excising the first node draining a tumor, as identified by a combination of radioactive tracer and dye. The procedure was made possible by the discovery of lymphoscintigraphy in 1977 and first described by Morton in 1992.[115] The goals of SLNB include accurate staging, enhancing regional disease control, identifying patients for adjuvant treatment regimens and improving survival. Ten year survival for sentinel node negative and sentinel node positive patients is 85.7% and 63.1%, respectively, making it the single most important predictive factor for survival.[116] Morton pioneered the procedure and dedicated his life to continued improvements in the SLNB procedure, right up until his death in 2014.[117]

Before the SLNB was common practice in patients with melanomas ≥1 mm Breslow depth, as advocated by the AJCC, many surgeons favored ELND as consensus was that this improved outcomes.[51] Physicians observed that lymph node metastasis often precedes more widespread metastatic disease, and hypothesized that removal of lymph nodes therefore may prevent systemic metastasis. The procedure was abandoned after four trials did not show survival benefit, although the fact was recognized that regional lymph node positivity is strongly associated with a worse prognosis.[9,11,118,119] As compared with patients who never developed nodal metastases after wide excision of the primary without ELND, the HR for death was 1.25 in patients with histologically positive nodes at ELND and 2.11 in patients who developed node metastases during follow-up and underwent a DLND.[9]

The SLNB procedure has revolutionized surgical treatment of melanoma as it has made the ELND largely obsolete, with much less morbidity.[7,115] During the procedure, the first lymph node or group of lymph nodes to drain lymphatic flow from the primary tumor site is identified using radioactive blue dye, 1% isosulfan blue with the addition of Tc99m sulfur colloid, and resected.[44] The radioactive tracer allows for creating a map of lymphatic drainage using lymphoscintigrapy before surgery and identifying the sentinel lymph node during surgery using a handheld scanner with a gamma sensor probe. The blue dye allows for visual identification of the sentinel lymph node and distinguishing lymphatic tissue from surrounding tissues. Using a combination of both tracer and dye garners higher identification rates than using either technique alone.[120] Drainage to more than one lymph node basin occurs in 15-27% of patients.[44] The excised tissue is examined postoperatively, as the sensitivity for melanoma detection has been found to be higher in formalin-fixed tissue as opposed to frozen tissue.[121] Frozen tissues provide a suboptimal morphology, requires embedding in paraffin which leads to unexamined sections and may lack the subcapsular region of the lymph node, which is where micrometastases often are found.[122]

The procedure is aimed at identifying occult lymph node mestastases. If the sentinel node is positive, patients will undergo CLND with removal of all lymph nodes in the basin, as identified in the NCCN guidelines.[123] The only exceptions to these are enrollment into a clinical trial and severe comorbidities precluding surgery.[123] The procedure is highly reliable, with the ability to find the sentinel lymph node in 96% of patients. SLNB is recommended in patients with tumors ≥1 mm thick.[51] The MSLT-I trial looked at wide local excision, nodal observation and TLND compared to wide local excision, SLNB and immediate lymphadenectomy for nodal metastases.[116] Ten year disease free survival was significantly improved in the SLNB arm in patients with intermediate thickness (1.20-3.50 mm, HR 0.76) and thick (>3.50 mm, HR 0.70) melanomas. There was no difference in melanoma-specific survival rates or OS after ten years.

Of the patients who do undergo CLND, additional positive lymph nodes are found in 20%.[122] In patients with melanoma ≤1 mm, the sentinel lymph node is only positive in 5% of cases. These observations led to the design of the MSLT-II trial, which randomized patients with a positive SLNB between CLND and ultrasound monitoring of the lymph node basin.[124] Ultrasound can detect metastases as small as 4 mm. The primary endpoint is melanoma-specific survival. Results are still awaited.

The optimal timing to perform the SLNB procedure after excisional biopsy has not been established, however, consensus is that the procedure should be done as soon as possible. A cohort study in 1015 patients did not identify time interval from primary excision to SLNB as a prognostic factor for survival after a follow-up of three years.[125] Morbidity of the SLNB is relatively limited, with wound infections, seroma, postoperative bleeding and erysipelas reported in 2% of patients and mild lymphedema in 6-11%.[126,127] CLND leads to a marked increase in lymphedema, with 7% of patients reporting symptoms after axillary node dissection and 64% after inguinal node dissection, and wound complications (infection, seroma, necrosis, hematoma) in 51% of patients with inguinal lymph node dissection.[126-128] TLND leads to a higher frequency of lymphedema and longer hospitalization as compared to CLND.[129]

Locoregional treatment

Patients with limited locoregional disease often experience relatively few symptoms, which has prompted physicians to look into alternative treatment modalities. Treatment modalities described in this population include: intralesional injection for limited intralymphatic metastases, (hyperthermic) isolated limb perfusion (HILP/ILP) and isolated limb infusion (ILI) for bulky disease limited to the extremities and percutaneous hepatic perfusion (PHP) for patients with isolated liver metastases.[32,105-107,130-141] Locoregional therapy has several advantages over systemic therapy, as local drug administration allows for delivery of an increased concentration of the agent and reduced systemic exposure, thereby both increasing efficacy and lowering toxicity.[106,142] These treatments can be repeated multiple times, depending on response and toxicity.

Intralesional injection was first described by Coley, who reported regression of locally advanced tumors after injection of mixed bacterial toxins in 1893.[143] The ideal agent will express a *bystander effect*, where uninjected distant lesions exhibit a response.[130,131] Since then, numerous agents have been tested, but the most promising data have been found for PV-10 and talimogene laherparepvec (TVEC).[106,131,136,144-147] The latter was FDA approved in 2015.[33]

PV-10 (rose bengal) is a xanthine dye which creates reactive oxygen by reacting with visible and ultraviolet light, thereby mediating phototoxic reactions and inducing autolysis in lysosomes of cancer cells, which selectively absorb the agent.[136,147] Its predecessor was first patented back in 1882 as a wool dye and the first clinical application of rose bengal was to combat ocular pneumococcal infection in 1914.[148] Other applications included use as an ophthalmic diagnostic agent, a marker for impaired liver function (now redundant) and a food dye. Its anti-carcinogenic effects were not discovered until the 1980s, when the Japanese Ministry of Health and Welfare decided to look into the safety of artificial food colorings. Akihiro Ito evaluated rose bengals tumorigenicity in mice and found, contrary to his expectation, dose-dependent survival increases for mice who received daily rose bengal after 82 weeks of exposure.[148]

TVEC is an oncolytic, immune-enhanced herpes simplex virus type 1, selectively infecting cancer cells and destroying the cells by direct effects on metabolic processes and inducing immune responses.[131] TVEC has shown an OS benefit as compared to GM-CSF (23.3 months vs. 18.9 months) in patients with stage IIIB/IIIC/IV-M1a cutaneous head and neck melanoma in its randomized phase 3 trial.[131] A retrospective subgroup analysis suggests that the results in melanomas of the head and neck may be even better.[131]

Regional therapies were first reported in the 1950's when open cannulation (i.e. placing a cannula in a blood vessel using an open surgical procedure, as opposed to percutaneous placement) and surgical control of the vessels were achieved to provide intra-arterial regional chemotherapy.[31] The first techniques described used open procedures, requiring a laparotomy for hepatic perfusion and an incision in the groin/axilla for HILP/ILP.[134,140,149] These techniques were later refined using a percutaneous approach for PHP and ILI, which decreased the duration of the procedures and associated morbidity and mortality.[32,106]

The extremities are particularly suitable for perfusion and infusion techniques as the vascular in- and outflow can be isolated using an extremity tourniquet. The systemic leak rate is less than 1%.[137] The chemotherapeutic agents most widely used in ILI and HILP are melphalan combined with tumor necrosis factor-α (TNFα) and dactinomycin.[132,133,135,141,150] Reported results for ILI demonstrate complete response (CR) rates ranging from 23% to 44% and partial responses (PR) from 27% to 56% with a median duration of response between 12-18 months.[139,151,152] ILP improves response rates as compared to ILI, but not PFS or OS.[153] Burden of disease is a prognostic factor for response.[134,140,149]

Although percutaneous perfusions have been described for other organs, the liver is specifically suitable for this approach because venous outflow into the systemic circulation for the entire liver is via the hepatic veins into the inferior vena cava IVC, vascular isolation of the liver can be achieved via balloon occlusion of the inferior vena cava. This also allows for filtering the chemotherapeutic agent with a veno-venous bypass before it reaches systemic circulation in order to limit systemic toxicity. PHP is especially important in uveal melanoma, where 95% of patients that develop metastatic disease will have liver metastases, which in 80% of cases will be the only site of distant disease. Even large tumors, covering more than 50% of the liver, can be treated this way.[106,137] The overall response rate (ORR) for this procedure is 60% and disease control rate is 90%.[154] A retrospective cohort reported a significantly improved PFS for PHP of 245 days, compared to 52 days for chemoembolization and 54 days for yttrium-90, however, it should be taken into account that not all patients are candidates for PHP and more (randomized) research is needed in this area.[155]

Adjuvant and neo-adjuvant treatment

Until recently, adjuvant (i.e. post-surgery) and neoadjuvant (i.e. pre-surgery) systemic therapies had not shown an improvement in survival. Up until 2015, high dose interferon-α during one year was the only adjuvant therapy approved by the FDA.[98,156]

A pooled analysis of all randomized trials involving high dose interferon-α (n = 4) conducted by the Eastern Cooperative Oncology Group (ECOG) showed a significant decrease in relapse free survival (RFS), but not OS. Study E1684 compared adjuvant high dose interferon-α to observation and reported a HR for recurrence-free survival (RFS) of 1.38 in favor of interferon-α. E1690 compared three groups; observation vs. low dose interferon-α (no benefit) and high dose interferon-α; the HR for RFS was 1.24 (non-significant). Neither study reported a statistically significant OS benefit. E1694 compared interferon-α to GMK vaccine and demonstrated both improved RFS (HR 1.33) and OS (HR 1.32). E2696 included three groups; GMK vaccine alone vs. GMK with either concurrent or sequential interferon-α. This study showed neither RFS nor OS improvement.[157]

The most extensive data on this topic, however, come from a meta-analysis of all randomized controlled trials published between 1990 and 2008 (n = 14) and involved 8122 patients. In these trials, 17 comparisons were made between interferon-α and a comparator. Disease free survival was improved in 10/17 comparisons (HR 0.82) and OS was improved in 4/14 comparisons (HR 0.89).[156]
Discontinuation rates of 37% have been reported because of toxicitcy.[158] Based on these data, the benefit of adjuvant treatment with interferon-α has always remained controversial.

In 2015, the FDA approved adjuvant use of ipilimumab for stage III melanoma patients based on a phase 3 trial conducted by Eggermont et al.[33,159] Patients who had undergone complete resection of stage III melanoma were randomized between 10 mg/kg ipilimumab and placebo. After a median follow-up of 5.3 years, RFS was 41% vs. 30% and 5-year survival was 65% vs. 54%, both statistically significant with approximately 25% risk reduction. However, over 40% of patients treated with ipilimumab in this study experienced a treatment-related grade 3-5 adverse event. This has placed concern over the routine use of ipilimumab 10mg/kg in a clinical setting. Ipilimumab has been directly compared to high dose interferon-α in the ECOG 1609 study, but the results have yet to be reported.

Regarding neoadjuvant therapy, small prospective studies with temozolomide, interferon-α, and biochemotherapy - a regimen containing multiple chemotherapeutic agents in combination with interferon-α - have demonstrated tumor burden reduction and occasional pathologic complete responses in resectable stage III patients. ORR's of 16% for temozolomide, and 26% for biochemotherapy were shown.[160-162] For high-dose interferon-α, an objective clinical response of 55% was reported.[162]

This landscape may change when results from trials using BRAF-targeted therapy and anti-PD1 therapy become available.

Systemic treatment in advanced melanoma

Systemic therapy is the primary treatment for patients with unresectable locoregional and metastatic melanoma. Prior to the recent therapeutic advantages, cytotoxic agents were the first choice for treatment in advanced melanoma. Chemotherapeutic agents used for melanoma treatment include dacarbazine, cisplatin, temozolomide, nitrosoureas (fotemustine, carmustine and lomustine) and taxanes (docetaxel and paclitaxel). ORR's range from 10-20%.[163] With the exception of dacarbazine, none of these have received a formal approval by the FDA.[33] Although the antimetabolite hydroxyurea received FDA approval as well, based on efficacy in combination with radiotherapy, consensus is that hydroxyurea does not show efficacy in metastatic melanoma as monotherapy.[34,35]
Out of the enlisted options, the two main drugs that have been used are dacarbazine and temozolomide. Dacarbazine was introduced in 1972 and FDA approved in 1976.[164] It yielded a response rate of 16% and the median OS was 4.5-6 months. Long term responses are extremely rare, with less than 2% of patients being alive after six years.[164,165] Although never formally approved, temozolomide has shown similar efficacy to dacarbazine and has the added advantages of crossing the blood brain barrier and being an oral agent.[166] While older regimens such as biochemotherapy have fallen out of favor due to associated toxicities and unclear benefit over other options, carboplatin plus paclitaxel is another regimen that has been used based on an ORR of 11% and median PFS of 17.9 weeks.[167] No chemotherapy regimen has demonstrated an improvement in overall survival.[163]
Targeted agents, such as BRAF and MEK inhibitors, selectively inhibit a mutated protein or activated pathway that is unique to the tumor, as opposed to chemotherapy, which targets all rapidly dividing cells. For the majority of mutations shown in Figure 4 (e.g. P13K, CDK4/6 and mTOR) targeted inhibitors exist, but so far these have not proven a benefit in melanoma.[87] Other mutations such as NRAS, KRAS and HRAS have not yet seen successful therapies specifically targeting the mutation.
The first BRAF inhibitor proceeding to phase 3 trials was sorafenib, which was added to a carboplatin/paclitaxel regimen.[167] Due to its low potency and low selectivity for BRAF V600E it failed to achieve its goals. Phase 3 randomized studies of BRAF inhibitors vemurafenib and dabrafenib vs. dacarbazine have shown objective response rates of 50-53% in metastatic BRAF V600E mutant melanoma patients.[37,38] Although responses seen using BRAF monotherapy can be dramatic, the majority of patients develop resistance, with a median PFS of five months in the earlier trials and seven to nine months in the later trials.[37-39,168] Like immunotherapy, BRAF inhibitors have also shown improved survival in melanoma patients, with 84% of vemurafenib patients alive after six months, compared to 64% of patients on dacarbazine. Resistance against BRAF inhibitors may be intrinsic or acquired. Most mechanisms lead to reactivation of the MAPK pathway or activation of PI3K/AKT/mTOR pathway. The addition of MEK inhibitors increases response rates to 68% and extends the median PFS to 9-13 months.[39,40,79,168] The importance of combination therapies becomes apparent when looking at the pathways in Figure 4, as melanoma develops resistance in >95% of patients treated with chemotherapy, interferon-α and IL-2 and >75-80% in patients treated with BRAF-targeted therapy or ipilimumab.[97,169,170] In the case of treatment with BRAF and MEK inhibitors, vertical blockade of the MAPK pathway increases efficacy and delays the onset of resistance.
As introduced in the paragraph 'A brief history in cutaneous melanoma' (page 11), another strategy has been to target the immune system to drive anti-tumor immune responses against melanoma tumors based on its established immunogenicity. The first systemic immunotherapeutic agents that demonstrated clear activity in patients with advanced melanoma were interferon-α and high dose interleukin-2 (IL-2), FDA approved in 1995 (as adjuvant therapy) and 1998, respectively. IL-2 has a response rate of 16% and interferon-α of 23%.[97,171] Both agents did improve long term survival in a small subset of patients (<5%), however, median survival still was only six and twelve months, respectively. [97,171]
The current decennium has seen a second wave of immunotherapies. Ipilimumab, an anti-CTLA-4 antibody, was FDA approved in 2011. It was the first drug to show an OS benefit for melanoma, though it was still limited by the low percentage of responders, with an ORR of 10-

12%. Hodi et al. showed a median OS of 10.0 months in 403 patients receiving ipilimumab plus glycoprotein 100, a peptide vaccine, vs. 10.1 months in 137 patients treated with ipilimumab alone, vs. 6.4 months in 136 patients treated with glycoprotein 100 alone (p = 0.003). No survival difference was found between the two ipilimumab-containing regimens.[36] Although the median PFS in the phase 3 trial was relatively short (2.9 months), the OS following ipilimumab treatment plateaus around 20% at three years, indicating a group of patients with long-term benefits.[170] Ten year follow-up data have now been reported and continue to show the 20% plateau.[170]

Pembrolizumab and nivolumab, both blocking the checkpoint molecule PD1, received FDA approval in 2014. Agents that act on PD1 and PD-L1 may only be the beginning of a renewed exploration into using the immune system as a tool against cancer. Of note, responses to immunotherapy cannot always be measured by using the traditional response evaluation criteria in solid tumors (RECIST), as pseudoprogression has been reported. Therefore, other response criteria such as immune-related response criteria (irRC) and immune-related RECIST (irRECIST) have been developed.[99,172]

Both pembrolizumab and the combination nivolumab/ipilimumab have shown improved PFS and OS when directly compared to ipilimumab.[41,43,173] Robert et al. reported a 12-month survival rate of 74% for pembrolizumab every two weeks, 68% for pembrolizumab every three weeks and 58% for ipilimumab in patients with unresectable stage III or stage IV melanoma who had received no more than one previous systemic treatment for advanced disease.[43] Larkin et al. reported a PFS of 11.5 months for nivolumab/ipilimumab, 6.9 months with nivolumab only and 2.9 months with ipilimumab only in previously untreated melanoma patients with unresectable stage III or stage IV melanoma.[173] ORR in frontline settings is 40-45% with nivolumab and pembrolizumab monotherapy and up to 60% with nivolumab/ipilimumab.[41,43,174] PD-1 and PD-L1 antibodies have shown efficacy in a range of tumor types, e.g. lung cancer and Merkel cell carcinoma, although response rates have not been as high as those seen in melanoma (33-40%).[41,43]

Other immunogenic treatment modalities in melanoma are tumor specific vaccines, which have been largely abandoned due to disappointing results, and tumor-infiltrating lymphocytes (TIL), which have shown promising results in phase 1/2 trials.[175] The presence of lymphocytic infiltrates within tumors is associated with better outcomes in several tumor types, including metastatic melanoma.[175] This has led to the development of autologous TIL, with lymphocytes being grown from resected lesions. Patients are treated with a lymphodepleting regimen and infused with TIL, followed by IL-2 to support the continued growth and activity of the infused TIL. The optimal regimen has not been established yet. Reported ORRs range from 21-72% is treated patients, but have to be interpreted with caution, as TIL cannot be grown for all patients included in trials, which introduces a bias in the reported outcomes.[175] Rosenberg et al. reported a 5-year survival rate of 93% in the 20/93 heavily pretreated patients who developed a complete response.[176] A phase 3 trial comparing TIL to ipilimumab in patients with unresectable stage IIIC or stage IV melanoma is in progress (NCT02278887).

The clinical development of checkpoint immunotherapies and targeted therapies has surely improved outcomes for melanoma patients. Median OS of 24 months or longer have now been achieved in clinical trials of dabrafenib plus trametinib and nivolumab plus ipilimumab.[79,173] In the nivolumab/ipilimumab phase 2 and 3 studies, median OS has not been reached.

Clinical challenge: melanoma brain metastasis

In absolute numbers, melanoma is the third most frequently metastasized cancer to the brain, after breast cancer and lung cancer.[76] Over a third of patients with advanced melanoma will develop brain metastases during the course of their disease, and even higher rates have been observed at autopsy.[177,178] Historically, the prognosis of patients with melanoma brain metastases (MBM) has been poor, with median OS ranging from 2-5 months from time of diagnosis.[177-181] Patients with solitary or oligometastatic disease amenable to surgery or stereotactic radiosurgery (SRS) typically have better survival than the general MBM population, with median OS reported from 7-10 months.[182-184] This is likely reflective of improved local MBM control and patient selection. The criteria for SRS are not set in stone; traditionally SRS was considered for patients with ≤3 MBM and no lesions >3 cm in diameter, but the current limit is more fluid and patient-specific.[76] When surgical resection or SRS are not an option, whole brain radiation therapy (WBRT) has been used, at the cost of neurocognitive deficits.

The molecular biology of MBM is not well understood and research has garnered conflicting data. Data on molecular markers have demonstrated an increased rate of MBM in patients whose disease harbor BRAF V600 and NRAS mutations, as well as shorter time to MBM with loss of PTEN/PI3K pathway activation - although this has been controversial with other reports showing no clear correlation between BRAF mutations and MBM incidence.[91,185-187] Systemic treatment of MBM comes with specific challenges and lack of response may be due to inadequate blood brain barrier penetration, drug efflux pumps, intrinsic resistance or protection against drug-induced apoptosis from specific cells in the brain microenvironment, such as astrocytes.[76] Phase 1 and retrospective data with BRAF-targeted therapy have shown that the brain is an important location for progression, with MBM development occurring in 20-43% patients at time of progression, thereby raising questions about the relationship between treatment regimens and MBM development, as addressed in chapter 8.[169,188,189]

Past systemic therapies have demonstrated limited benefit in patients with active MBM. Most chemotherapeutic agents have minimal to no activity in treating MBM. Among those that have modest activity, such as temozolomide and fotemustine, the objective MBM response rates have ranged from 7% to 12%.[190-195] The addition of WBRT has provided marginal benefit to patients. Similarly disappointing results have been seen in systemic therapy with high-dose interleukin-2 (IL-2), with a combined intra- and extra-cranial response rate of 6% in patients with active MBM.[196,197] Furthermore, chemotherapy and IL-2 regimens have demonstrated low overall success in preventing the development of MBMs. In a prospective study designed to evaluate the MBM incidence between cisplatin/temozolomide/IL-2 and cisplatin/dacarbazine/IL-2, 49% of assessable melanoma patients developed disease located in the central nervous system and there was no significant difference between the arms.[198] Interestingly, at least two

Table 1 - Historical landmarks in melanoma

±2625 BC	Imhotep documents the first known case report of cancer
±400 BC	Hippocrates documents the first known case report of melanoma
1787	John Hunter reports a surgical removal of melanoma
1812	René Laennec first describes melanoma as a disease entity
1857	William Norris published the first melanoma case series in the English literature
1840	Samuel Cooper notes that early surgical removal of melanoma 'is the only chance for benefit'
1892	Herbert Lumley Snow advocates for 'anticipatory gland dissection' before gland enlargement
1907	William Handley advocates for wide excision
1958	Edward Krementz publishes first case series of isolated limb perfusion with melphalan
1967	FDA approval of hydroxyurea for advanced melanoma
1968	Donald Morton publishes first series of patients treated with BCG vaccination
1969	Ion Gresser describes the role of interferons in antitumor immunity
1969	Wallace Clark notes the pathologic heterogeneity of melanoma and levels of invasion that correlate with prognosis
1970	Alexander Breslow describes the relationship between tumor thickness and prognosis
1970	Donald Morton publishes the first successful clinical application of immunotherapy directed against a metastatic human cancer
1974	Donald Morton describes presence of melanoma antigens
1976	FDA approval of dacarbazine for advanced, metastatic melanoma
1985	John Kirkwood initiates high dose interferon studies in patients with high risk of relapsed melanoma
1988	First version of the AJCC staging system for melanoma
1990	Ferdy Lejeune publishes on locoregional use of tumor necrosis factor
1992	Donald Morton publishes technical details regarding the use of intraoperative sentinel lymph node mapping
1992	Danielle Lienard reports success with a combination of TNF-α, interferon-γ and melphalan in isolated limb perfusion
1996	The FDA approves high dose interferon for adjuvant treatment of high-risk melanoma
1998	The FDA approves high dose bolus IL-2 for advanced, metastatic melanoma
2002	Helen Davies describes a high frequency of the BRAF mutation in melanoma
2005	Boris Bastian publishes the first report that melanomas are genetically heterogenous
2006	Donald Morton publishes evidence in favor of sentinel lymphadenectomy with early nodal dissection
2011	The FDA approves three drugs; pegylated interferon for high-risk resected disease, ipilimumab for advanced, metastatic disease and vemurafenib for advanced, metastatic disease
2013	The FDA approves two drugs: trametinib and dabrafenib for BRAF mutated unresectable or metastatic melanoma
2014	The FDA approves pembolizumab and nivolumab for unresectable or metastatic melanoma
2015	The FDA approves talimogene laherparepvec for unresectable recurrent melanoma and cometinib for BRAF V600E or V600K mutated melanoma and ipilimumab for adjuvant treatment of stage III resected melanoma
2017	>600 active trials in melanoma on clinicaltrials.gov

Adapted and updated from Lee et al.[7]

retrospective studies have shown that patients demonstrating extracranial disease response to a systemic therapy have longer survival from MBM diagnosis.[179]

With regards to BRAF-targeted therapies and immune checkpoint therapies, the majority of patients enrolled on the registration protocols did not have a history of MBM and all patients were required to have prior MBMs treated with surgery and/or SRS. The effects of these therapies in MBM populations have been subsequently studied in smaller patient groups. Phase 2 studies of immuno-oncology and BRAF agents conducted in patients with active MBM have shown lower clinical activity than in non-MBM populations. These include objective MBM responses from as low as 5-22% with ipilimumab and pembrolizumab monotherapies, to as high as 39% with BRAF-targeted therapy; median OS ranged from 3-7 months from time of diagnosis for BRAF and ipilimumab studies, but has not yet been reached in the phase 2 pembrolizumab study after a median follow-up of 11.6 months.[199-201] Despite these results, retrospective data has suggested that survival outcomes are much improved when MBM patients have been managed with SRS and immunotherapy (ipilimumab) with median OS ranging from 12.4 to 21 months.[202-204] Trials are ongoing with dabrafenib plus trametinib and nivolumab plus ipilimumab in patients with active MBM.

Up to 60% of brain metastasis patients die of extracranial disease progression.[205] This suggests that MBM prevention and/or better control of disease in MBM patients could lead to improved outcomes, particularly with newer, more effective therapies. Real world data in large patient groups treated with recently approved therapies, which can be found in this dissertation, are largely missing.

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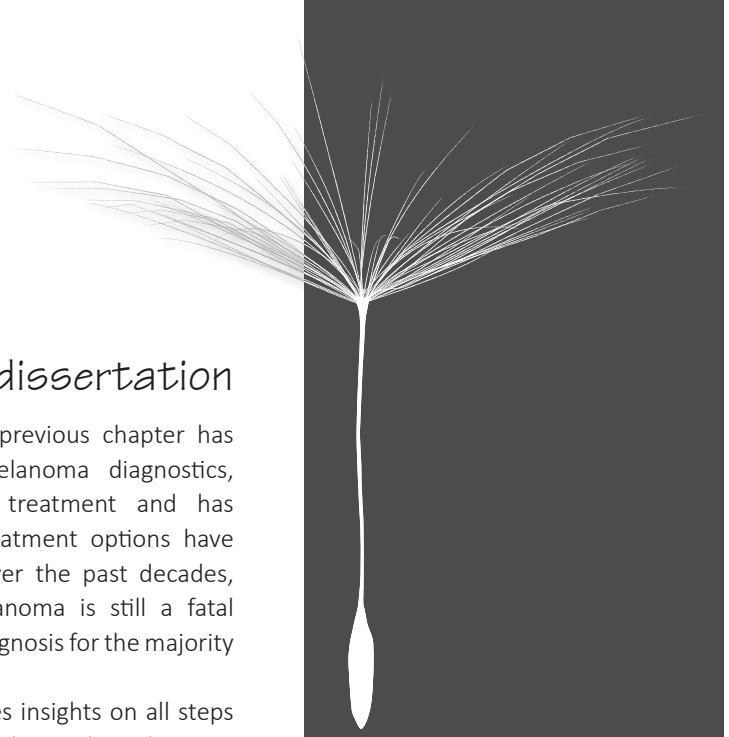
2 Outline of the dissertation



Outline of the dissertation

The introduction in the previous chapter has provided insights in melanoma diagnostics, biology, prognosis and treatment and has shown that although treatment options have significantly expanded over the past decades, once disseminated, melanoma is still a fatal disease with a somber prognosis for the majority of patients.

This dissertation generates insights on all steps of the treatment of advanced melanoma. It starts out with answering the important question whether sentinel lymph node biopsy leads to more intralymphatic metastases, then gives an update on locoregional treatment of melanoma, both infusion/perfusion techniques and intralesional treatment, and generates data for a potential neoadjuvant strategy with BRAF inhibitors. Lastly, this dissertation will investigate the effect of the recent additions to systemic treatments in melanoma brain metastasis and the resulting changes in prognosis. These insights aim to assist in clinical decision making.

Part I - Locoregional treatment developments in advanced melanoma

The relationship between sentinel lymph node biopsy and intralymphatic metastases is investigated and regional and intralesional therapies are reviewed.

- **Chapter 3**
  Is there a relation between type of primary melanoma treatment and the development of intralymphatic metastasis? A review of the literature
- **Chapter 4**
  Regional therapy in metastatic melanoma: an update on minimally invasive intra-arterial isolated limb infusion and percutaneous hepatic perfusion
- **Chapter 5**
  Intralesional therapy for metastatic melanoma

Part II - BRAF treatment in advanced melanoma

The potential use of BRAF-targeted therapy as a neoadjuvant strategy is described and long term effects of BRAF-targeted therapy are reviewed, which is increasingly relevant as more long term survivors who continue on BRAF therapy are identified.

- **Chapter 6**
  BRAF inhibition for advanced locoregional BRAF V600E mutant melanoma: a potential neoadjuvant strategy
- **Chapter 7**
  Long-term effects of BRAF inhibitors in melanoma treatment: friend or foe?

Part III - Developments in melanoma brain metastasis

One of the largest cohorts of melanoma brain metastasis patients in current literature is identified and prognostic factors, impact of new treatments and survival are investigated.

- **Chapter 8**
  Improved survival of patients with melanoma brain metastases in the era of targeted BRAF and immune checkpoint therapies

This dissertation will finish up with a discussion of the reported research results and expected future developments. A summary is provided in English and Dutch. The appendices include the curriculum vitae of the author, acknowledgements and the author's list of publications.

part I

Outline of the dissertation

3

Is there a relation between type of primary melanoma treatment and the development of intralymphatic metastasis? A review of the literature

Abstract

Background
Intralymphatic metastases (ILM) originate from tumor cell emboli entrapped in dermal lymphatics between primary tumor and regional lymph node basin. Because of this origin, sentinel lymph node biopsy (SLNB) might increase ILM by restricting lymph flow.

Methods
Pubmed, Embase, Cochrane and Medline were searched for articles on ILM between 1980 and September 2014. ILM Incidences were calculated after wide local excision (WLE), excision with elective lymph node dissection (ELND) or therapeutic lymph node dissection (TLND), WLE with SLNB with or without completion lymph node dissection (CLND) and delayed lymph node dissection (DLND) for patients developing nodal metastasis during follow-up.

Results
In 36 studies, 14,729 patients underwent WLE, 1,682 patients WLE/ELND, 362 patients WLE/DLND and 11,201 patients WLE/SLNB. On meta-analysis, ILM occurrence was 3.4% (95% CI 2.8-4.2%). ILM occurred most frequently in the WLE/DLND group (5.5%, 95% CI 3.5-8.7%), followed by WLE/ELND (4.7%, 95% CI 3.1-7.0%), WLE/SLNB (4.5%, 95% CI 3.5-5.7%) and WLE alone (1.9%, 95% CI 1.4-2.7%). 1,330 SLNB+ patients were identified and 5,783 SLNB-patients. For these groups, on meta-analysis, ILM recurrence was 13.2% (95% CI 10.8-16.2%) and 3.4% (95% CI 2.5-4.5%), respectively ($p = 0.01$).

Conclusion
In this review SLNB is associated with an increase of ILM with an incidence of 1.9% for WLE vs. 3.4% for WLE/SNLB. Selection bias in this review cannot be excluded. However, ILM occur four times more frequently after SLNB+ than SLNB-procedures and more often after SLNB+/CLND than WLE/DLND or WLE/ELND. ILM should therefore be viewed as a biomarker of aggressive primary disease.

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Introduction

The behavior of cutaneous melanoma is notoriously unpredictable. Five-year survival rates deteriorate as stage progresses. For stage IA, IB, IIA, IIB, and IIC these survival rates are 97%, 92%, 81%, 70% and 53%, respectively. Five-year survival for locoregional metastasis is 78% (stage IIIA), 59% (stage IIIB) and 40% (stage IIIC).[1] Once melanoma has metastasized distantly survival is around 15-20%, although these rates are expected to improve upon the recent introduction of BRAF-targeted drugs, checkpoint inhibitors and new generation immunotherapies[2-9] Long-term follow-up reveals that ulceration and sentinel lymph node status are the strongest predictors for survival.[10,11]

The concept of incidence of locoregional metastases increasing with tumor thickness was recognized decades ago.[12-14] Previously, in-transit metastases (ITM) and satellite lesions (SL) were considered different entities, but The American Joint Committee on Cancer (AJCC) has classified both ITM and SL since 2002 as intralymphatic metastases (ILM).[15] Historically, SL have been defined to reside within centimeters of the primary tumor location and ITM in the pathway between primary site and regional lymph node basin. The leading hypothesis is that both originate from tumor cell emboli entrapped in dermal lymphatic vessels between primary tumor and regional lymph node basin.[16,17] The appearance of ILM automatically upstages a patient's disease into stage IIIB/IIIC, decreasing 5-year survival to 59% and 40%, respectively.[1] Survival rates for patients with SL alone, SL/ITM, or ITM are identical and similar to that of patients with nodal disease.[18] Scar recurrence, 'true local recurrence', differs in pathophysiology, as these develop from residual cells of the initial melanoma, a result of false-negative margins or microsatellites.

Curative treatment for primary melanoma remains surgery (wide local excision, WLE).[2,19] Four prospective additional elective lymph node dissection (ELND) trials showed no impact on survival.[20-24] ELND has become redundant after the introduction of the sentinel lymph node biopsy (SLNB) in 1992, which preserves its diagnostic advantage with less morbidity.[21-23,25-27] Patients with a positive SLNB undergo a completion node dissection (CLND). The MSLT-I study showed a small but significant disease-free and melanoma-specific survival benefit in patients with intermediate thickness melanoma (1.2-3.5 mm) and nodal disease following early treatment.[28] Most notably, a melanoma-specific survival improvement of 20% was reported for patients with intermediate thickness melanoma undergoing SLNB as opposed to observation, although the MSLT-I did not show improvement in recurrence free, distant metastasis free and melanoma specific survival for the entire population. The MSLT-II study will answer in the near future whether a CLND is indeed indicated after a positive SLNB.[29,30] Other treatment modalities have included therapeutic lymph node dissection (TLND), for metastatic nodal disease at the time of diagnosis, and delayed lymph node dissection (DLND), for patients developing metastatic nodal disease.[31]

SLNB in addition to WLE alone has been suspected of causing ILM by inducing lymphatic stasis or entrapment of melanoma cells.[32,33] Pathophysiology on which this hypothesis is built is that the lymph flow from the skin reaches the nodal basin within minutes, with melanoma cells still in lymphatic channels en route to the lymph node basin at the time of SLNB or nodal dissection.[33,34] Estourgie et al. published a fourfold risk of ITM recurrence in SLNB positive patients as compared to SLNB negative patients, thereby raising the question whether surgical treatment of the regional lymph node basin can be responsible for ITM, although the same research group refuted this finding in a larger population.[35,36] Although various authors have studied this phenomenon, most notably Morton et al. in the aforementioned MSLT-I trial and van Poll et al. using data of the Melanoma Institute Australia, a definite answer as to whether the incidence of ILM should be attributed to unfavorable primary tumor characteristics alone or is increased by the SLNB procedure by means of a review of all available data has not yet been published.[10,16,28,37,38]

The objective of this review was to provide an extensive body of evidence, answering the question whether ILM frequencies increase after performing SLNB.

Methods

Pubmed, Embase, Cochrane Library and Medline were searched for articles using the terms 'melanoma' and 'recurrence' or 'in-transit metastasis' or 'ITM' or 'SL' or 'intralymphatic metastasis' or 'local recurrence' or 'satellite' or 'sentinel node' or 'survival' between January 1980 and September 2014. Articles were excluded if they had not been written in English, if they did not distinguish between a local recurrence and ILM, if incidence for ILM as a first recurrence (FR) was not reported, if studies exclusively reported on SLNB- or SLNB+ or if treatment strategy was unclear. Duplicates, case reports, letters to the editors and case series were excluded. Data regarding ILM as FR derived from our institution's SLNB database (UMCG database) were added to the review.

ITM was classified as recurrent melanoma in the pathway between primary melanoma location and the regional nodal basin, with the lesion more than two or five centimeters from this location, depending on the definition used in the article. All other cutaneous and subcutaneous metastases between the re-excision scar and the location of ITM were classified as SL. As consensus is now that ITM and SL are the same entity, all ITM and SL were combined into one value, 'ILM'.

For all included articles the number of patients with ILM as first recurrence (FR) were calculated per treatment group: for WLE alone, for WLE with ELND, WLE and DLND or TLND and WLE with SLNB. The last group was stratified into tumor-negative SLNB (SLNB-) patients and tumor-positive SLNB (SLNB+) patients undergoing CLND. When assessing risk of ILM as FR, WLE was compared to the WLE/SLNB- group. WLE/SLNB+ was compared to WLE/DLND, WLE/ELND and WLE/TLND groups. As only SLNB+ patients undergo additional CLND, this division groups together the most similar procedures regarding interruption of lymph flow. Additional study characteristics were collected: study design, number of patients, mean/median Breslow thickness, age at diagnosis, and melanoma ulceration status.

Statistical analysis

For a comprehensive review of the data, all data were summarized in tables and analyzed using version 18 SPSS, (IBM, Chicago, Illinois, USA). Descriptive statistics were used to calculate frequencies of ILM for the different treatment strategies. Chi-square tests were used to check for significant differences.

Subsequently, all studies were assigned a weight based on the amount of included patients and entered into a meta-analysis. Meta-analyses were performed stratified for treatment, SLNB results and anatomical localization of the primary tumor. Proportions of ILM and the corresponding 95% CI were calculated and entered in a datasheet. Meta-analyses were performed with the 'metan' module using STATA/SE version 12.0 (StataCorp, College Station, Texas, USA) with the original data as reported in the studies. Pooled ILM proportions and their 95% CI were calculated using a random effects model.

Results

Study characteristics

19,620 studies were identified and assessed according to the inclusion criteria. 36 studies with a total of 33,622 patients were included for analysis (Table 1), including our ongoing academic medical center database (UMCG database). Six studies were excluded because they exclusively reported on SLNB- or exclusively on SLNB+ patients (n = 684 patients).[11,39-43] Median follow-up ranged from >12 months-11 years. Fifteen out of 36 studies reported mean Breslow depth and six reported exclusively median Breslow depth. One study reported Breslow depth using incremental depths.[44] Melanoma ulceration status was reported in 23 studies; in 15 of those data were only available for part of the population. Twelve studies provided treatment/recurrence data on WLE (14,729 patients), 5 on WLE/ELND (1,682 patients), 1 on WLE/DLND (362 patients) and 18 on WLE/SLNB (11,201 patients). For the remaining 5,648 patients in seven studies, treatment was not specified. No study reported outcomes exclusively for TLND. In 23 of the 36 included studies a clear definition of ITM/SL was not provided. ITM was defined as (sub)cutaneous disease recurrence between locoregional lymph node basin and 2, 3 or 5 cm from the original scar in n = 5, n = 1 and n = 4 studies, respectively. The remaining three studies defined ILM as recurrence within the pathway of lymphatic drainage, between scar and regional nodal basin, and between tumor and nodes, respectively. Seven out of 36 studies distinguished SL from ITM; out of these, two studies defined SL and LR as the same entity.[13,16,35,45-48]

Table 1 - Characteristics of included studies

No	Author	Year	No. patients	Age	Follow-up (median)	Breslow (mm, mean)	Ulceration	No. of ILM	% ILM	No. SLNB patients	SN+		SN-	
											Pts	ILM	Pts	ILM
1	Bagley[12]	1981	103	NR	>5 years (mean)	NR	NR	5	4.9	NR	NR	NR	NR	NR
2	Janoff[14]	1982	122	NR	6.1 years (mean)	NR	NR	8	6.6	NR	NR	NR	NR	NR
3	Roses[13]	1983	658	NR	44.8 months (mean)	NR	NR	15	2.3	NR	NR	NR	NR	NR
4	Veronesi[59]	1991	612	0-20: 6 21-40: 217 41-50: 159 51-65: 230	90 months (mean)	1.0	NR	4	0.65	NR	NR	NR	NR	NR
5	Heenan[45]	1992	482	NR	5 years (mean)	NR	NR	7	0.62	NR	NR	NR	NR	NR
6	Gadd[60]	1992	1019	56	NR	NR	NR	89	8.7	NR	NR	NR	NR	NR
7	Fusi[44]	1993	1090	NR	84 months	<0.75 6% <2.25 38% >2.25 56%	NR	20	1.8	NR	NR	NR	NR	NR
8	Martini[61]	1994	840	53.5	48 months	2.3	NR	24	2.9	NR	NR	NR	NR	NR
9	Karakousis[62]	1996	742	48.9	92 months (mean)	2.0	Present in 25%; NR 17	47	6.3	NR	NR	NR	NR	NR
10	Johnson[63]	1999	306	50.6	85 months (mean)	NR	NR	1	0.3	NR	NR	NR	NR	NR
11	Borgstein'16	1999	258	NR	27 months	1.5 (median)	NR	15	4.3	258	53	NR	205	NR
12	Cohn-Cedermark[46]	1999	2493	NR**	11 years	NR	1.1-2.7 (median)**	49	1.97	NR	NR	NR	NR	NR
13	Cohn-Cedermark[64]	2000	989	51-52 (median)	11 years	1.2 (median)	NR	9	0.9	NR	NR	NR	NR	NR
14	Chao[65]	2002	1183	52.0	16 months	NR	Present in 30%; NR 56	14	1.2	NR	NR	NR	NR	NR
15	Goydos[66]	2003	175	NR	NR	NR	NR	14	8.0	175	102	14	73	0
16	Estourgie[35]	2003	250	NR	72 months	2.7	Present in 32%; NR 3	32	10.8	250	60	14	190	18
17	Borgognoni [67]	2004	375	55.3	35 months	NR	NR	7	1.9	375	75	1	300	6
18	Macripo[68]	2004	274	51 (median)	2.9 years	1.9 (median)	Present in 8%	10	3.65	274	46	2	228	8
19	Thomas[69]	2004	900	57-58	60 months	3.1 (median)	Present in 33%; NR 125	17	1.9	NR	NR	NR	NR	NR

Table 1 - Characteristics of included studies (continued)

No	Author	Year	No. patients	Age	Follow-up (median)	Breslow (mm, mean)	Ulceration	No. of ILM	% ILM	No. SLNB patients	SN+		SN-	
											Pts	ILM	Pts	ILM
20	Berk[70]	2005	260	55	29 months	2.3	Present in 25%; NR 33	3	1.15	260	39	1	221	2
21	Duprat[71]	2005	240	51 (median)	27.8 months	1.6 (median)	Present in 30%	10	4.17	240	42	NR	198	NR
22	Nathansohn[72]	2005	141	53	41 months	NR	Present in 26%; NR 30	9	6.4	NR	NR	NR	NR	NR
23	Kang[20]	2005	4412	NR	NR	NR	Present in 9%; NR 45.9%	77	1.7	1016	110	9	906	28
24	Van Poll[47]	2005	2018	57	44 months (mean)	2.4	Present in 26%; NR 258	54	2.7	754	102	7	652	11
25	Pawlik[10]	2005	1395	51	46.8 months	1.5 (median)	Present in 21%	86	4.9	1395	234	28†	1136	40
26	Van Akkooi[73]	2006	262	NR	23.3 months	2.8	Present in 28%	11	4.2	262	77	7	185	4
27	Cecchi[74]	2006	111	53 (median)	31.5 months	NR	Present in 32%; NR 1	4	3.6	111	17	3	94	1
28	Kretschmer[75]	2006	328	60 (median)	40 months	2.7	Present in 34%; NR 16	25	7.6	NR	NR	NR	NR	NR
29	Dalal[76]	2007	1046	56 (median)	36 months (mean)	2.5	Present in 28%; NR 142	50	4.8	1046	163	23	883	27
30	Roulin[77]	2008	327	54	33 months	2.2	Present in 27%	20	6.1	327	74	10	253	10
31	UMCG database	2013	589	53	64.6 months	3.0	Present in 35%; NR 10	45	6.1	588	177	30	411	15
32	v/d Broek[78]	2013	305	51	>12 months	1.6	Present in 15%; NR 20	10	3.3	305	54	4	251	6
33	Ribero [79]	2013	1693	55.3-57.8 (median)**	4.8 years	1.41-2.21***	Present in 82%	92	5.4	656	NR	NR	NR	NR
34	Spillane[49]	2014	1704	<50 632 >50 1072	69 months	NR	Yes 549; No 826; NR 329	127	7.5	NR	NR	NR	NR	NR
35	Martin[50]	2014	80	49.9-50.9**	19.1-33.9 months**	2.86-3.11**	Present in 53%	12	15	NR	NR	NR	NR	NR
36	v/d Ploeg[80]	2014	5840	56.1-60.2**	42 months	2.33-2.47 ††	Present in 25%; NR 781	146	2.5	2909	394	NR	2515	NR

Age and Breslow depth are given as means unless otherwise reported. NR: Not reported; classified as number of patients; ILM: intralymphatic metastases; SLNB: sentinel lymph node biopsy

** No. of patients for whom SLNB data are available*

*** Values separately given for two different patient groups; median 1.1 mm for patients without recurrence and median 2.7 mm for patients with recurrence*

**** Values separately given for two different patient groups; mean 1.4 mm for patients with regression and mean 2.2 mm for patients without regression*

†SLNB status was split out for 68/86 ILM patients

†† Values separately given for two different patient groups; mean 2.3 mm for patients in observation group and mean 2.5 mm for patients in SLNB group

ILM data review

ILM occurred most frequently in the WLE/DLND group (20/362 patients, 5.5%), followed by WLE/ELND (75/1,682 patients, 4.5%), WLE/SLNB (both SLNB+ and SLNB-) (474/11,201 patients, 4.2%), and WLE alone (285/14,729 patients, 1.9%). For the remaining 5,648 patients, the occurrence of ILM was not specified according to treatment method. This group includes Spillane et al. and Martin et al., who did provide the amount of patients undergoing SLNB, but did not differentiate recurrence rates for CLND/DLND/TLND and CLND/TLND, respectively (Table 2).[49,50]

Of the 11,201 patients undergoing SLNB, ILM was split out according to tumor status in 6,913 patients. Of the SLNB+ group 153/1,330 patients (11.5%) developed an ILM as FR versus 176/5,783 patients (3.0%) in the SLNB-group. Differences in distribution between the four treatment modalities and differences between SN- and SN+ were statistically significant. ILM as FR after WLE was significantly lower than after WLE/SLNB, WLE/ELND and WLE/DLND (all $p < 0.001$). ILM was significantly lower after WLE/SLNB- compared to WLE/SLNB+ ($p < 0.001$, Table 3).

Meta-analysis

After review of the data a meta-analysis was performed, with weight assigned to studies based on the amount of included patients. The overall ILM incidence was 3.4% (95% CI 2.8-4.2%). In the meta-analysis, outcomes were similar to the review data with ILM occurring most frequently in the WLE/DLND group (5.5%, 95% CI 3.5-8.7%), followed by WLE/ELND (4.7%, 95% CI 3.1-7.0%), WLE/SLNB (both SLNB+ and SLNB-) (4.5%, 95% CI 3.5-5.7%) and WLE alone (1.9%, 95% CI 1.4-2.7%, Table 3/Figure 1). Of the 11,201 patients undergoing SLNB, ILM was split out according to tumor status in 6,913 patients. For the 6,913 patients whose SLNB outcome status was reported, ILM recurrence was higher than for the 11,201 patients, i.e. 5.8% (95% CI 4.1-8.3%). For SLNB+ patients, ILM occurrence was higher (13.2%, 95% CI 10.8-16.22%) than for SLNB-patients (3.4%, 95% CI 2.5-4.5%, Figure 2).

The WLE group had significantly less ILM recurrence than the SLNB group ($p = 0.02$), but not than WLE/ELND and WLE/DLND ($p = 0.21$ and $p = 0.49$, respectively). SLNB-patients had less recurrence than SLNB+ patients ($p = 0.01$) (Table 3).

Table 2 - Reviews classified by treatment, sorted by Brewslow thickness, for available studies

Author	Year	No. patients	No. of ILM	Percentage ILM	Breslow (mm, mean)
	WLE (n = 7,308)				
Veronesi[59]	1991	612	4	0.65	1.0
Van Poll[47]	2005	1035	26	2.51	1.8
Martini[61]	1994	840	24	2.85	2.3
v/d Ploeg[80]	2014	2931	51	1.74	2.3*
UMCG database[48]	2013	1	0	0.00	3.0
Cohn-Cedermark[64]	2000	989	9	0.91	1.2 (median)
Thomas[69]	2004	900	17	1.89	3.1 (median)
	WLE + ELND (n = 609)				
Karakousis[62]	1996	380	27	7.11	2.0
Van Poll[47]	2005	229	10	4.37	3.2
	WLE + DLND (n = 362)				
Karakousis[62]	1996	362	20	5.52	2.0
	WLE + SLNB (n = 8,868)				
v/d Broek[78]	2012	305	6	2.0	1.6
Van Poll[47]	2005	754	18	2.39	1.9
Roulin[77]	2008	327	20	6.12	2.2
Berk[70]	2005	260	3	1.15	2.3
Dalal[76]	2007	1046	50	4.78	2.5
v/d Ploeg[80]	2014	2909	95	3.27	2.5*
Estourgie[35]	2003	250	27	10.80	2.7
Van Akkooi[73]	2006	262	11	4.20	2.8
UMCG database	2013	588	45	7.65	3.0
Duprat[71]	2005	240	10	4.17	1.6 (median)
Pawlik[10]	2005	1395	86	6.16	1.5 (median)
Borgstein[16]	1999	258	11	4.26	1.5 (median)
Macripo[68]	2004	274	10	3.65	1.9 (median)

NR: not reported, classified as number of patients; ILM: intralymphatic metastases; WLE: wide local excision; ELND: elective lymph node dissection; DLND: delayed lymph node dissection; SLNB: sentinel lymph node biopsy

** Separate values given for separate treatment groups*

Figure 1 - Pooled percentage of ILM according to treatment

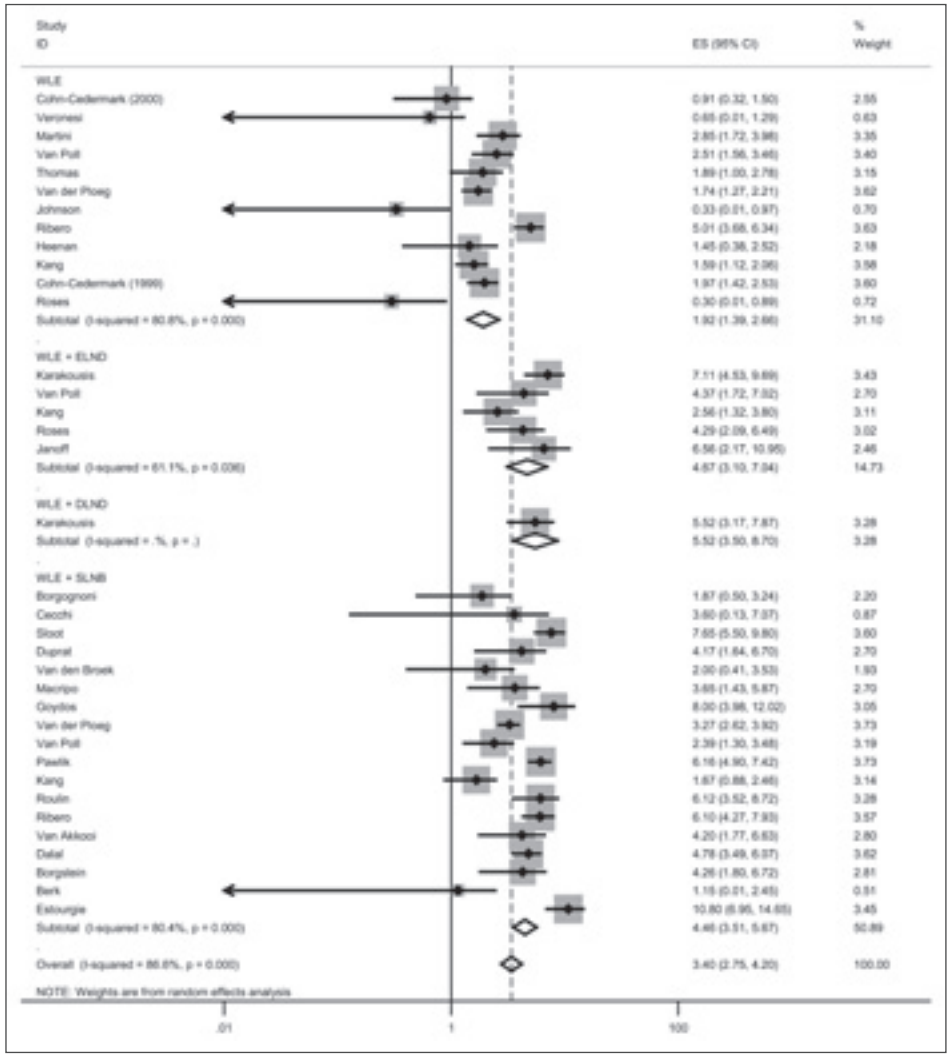

Figure 2 - Pooled percentage of ILM for according to SLNB positive or negative result

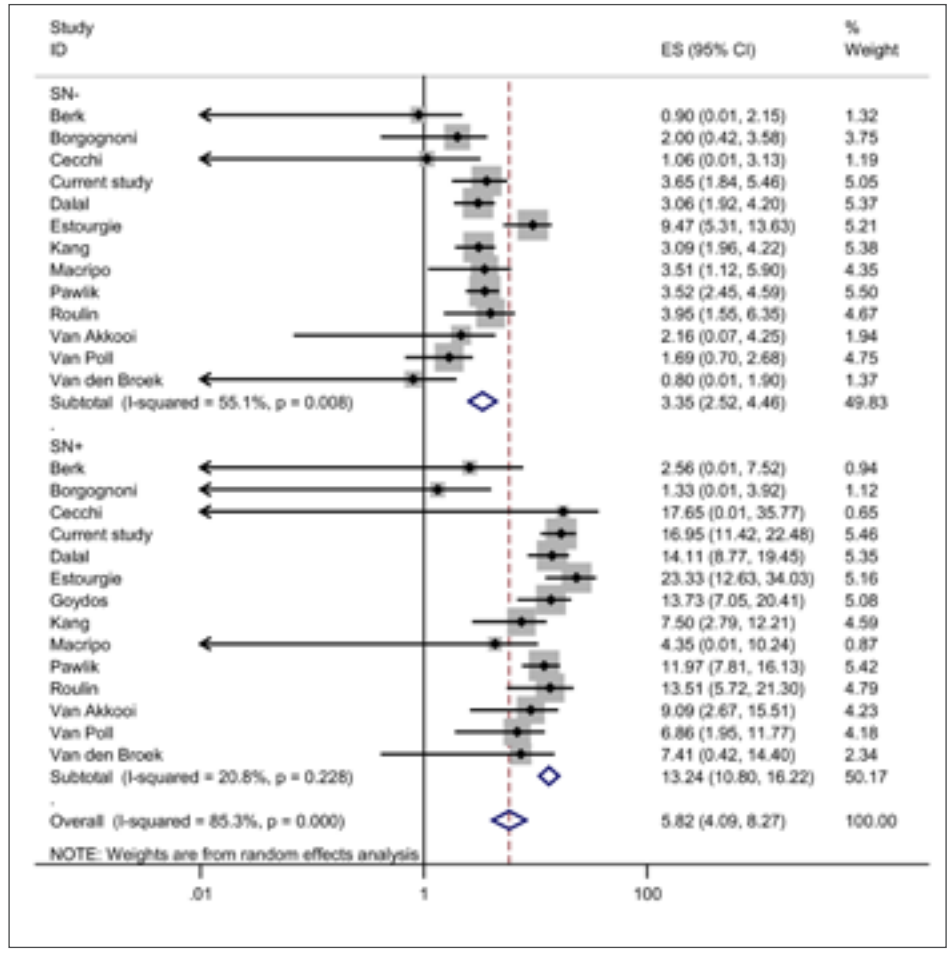

Table 3a - Pooled ILM estimates (%) from the meta-analyses, according to treatment, as shown in Figure 1 and 2

Treatment	Pooled value from meta-analyses		
	Estimate (%)	**95% CI**	***p*-value**
WLE	1.92	1.39-2.66	Reference value
WLE + ELND	4.67	3.10-7.04	0.21
WLE + DLND	5.52	3.50-8.70	0.49
WLE + SLNB	4.46	3.51-5.67	0.02
SN-	3.35	2.52-4.46	Reference value
SN+	13.24	10.80-16.22	0.01

Table 3b - Total number of ILM in the treatment groups for the initial treatments, review data*

Treatment	Number of ILM			
	Total	**ILM (%)**	**No ILM (%)**	***p*-value**
WLE	14,729	285 (1.9)	14,444 (98.1)	
WLE + ELND	1,682	75 (4.5)	1,607 (95.5)	
WLE + DLND	362	20 (5.5)	342 (94.5)	
WLE + SLNB	11,201	474 (4.2)	10,727 (95.8)	*p*-value four groups: < 0.001
SN-	5,783	176 (3.0)	5,607 (97.0)	
SN+	1,330	153 (11.5)	1,177 (88.5)	SN- and SN+: $p < 0.001$

NR: not reported, classified as number of patients; ILM: intralymphatic metastases; WLE: wide local excision; ELND: elective lymph node dissection; DLND: delayed lymph node dissection; SLNB: sentinel lymph node biopsy
** Stratified for SN- and SN+, review data. p-value for differences in distribution (Chi2)*

Discussion

Background

In this review, 33,622 melanoma patients from 36 studies were analysed to establish whether performing SLNB on melanoma patients in addition to WLE alone leads to an increase in ILM. This is an ongoing field of discussion in the literature. In fact, Read et al. recently published one of the largest databases so far (n = 11,614) where 505 patients developed ILM as a recurrence at any time during follow-up.[51] ILM percentages were 4.7% and 21.6% for SLNB- and SLNB+ patients, respectively. Numbers were not specified for the 190 patients who developed ILM as FR, which explains partly why the numbers are higher than in our study

Critics of SLNB have argued that as of yet there is no agreement on adjuvant therapy for node-positive patients and that only 20% of the patients undergoing SLNB will have a positive node.[52] However, nowadays there are new approaches available with targeted and/or immunotherapies that may lead to new adjuvant strategies.[53,54] The argument that no randomized controlled studies have shown a survival advantage for SLNB in node-positive patients has become partly redundant upon publication of the MSLT-I, which shows a (small, but significant) survival advantage for a selective group of patients, i.e. patients with an intermediate thickness melanoma and positive SLNB. Proponents advocate that SLNB is a procedure with a relatively low morbidity and that the current false-negative rate for SLNB performed in reputable institutes is <6%, declining further as experience progresses [55,56]

Results

Based on the results of our meta-analysis, the overall incidence of ILM as FR was 3.4%. Patients who did not undergo any lymph node dissection had the lowest incidence, with 1.9% of patients having ILM recurrence after WLE and 3.4% after SLNB-. ILM occurrence after WLE/DLND and WLE/ELND was slightly higher (4.7 and 5.5%, respectively), but incidence spiked after SLNB+/CLND at 13.2%. For TLND, insufficient data were available. Differences in ILM occurrence between WLE and WLE/SLNB groups were statistically significant, leading to the conclusion that a sentinel lymph node biopsy alone is associated with an increase in the risk of ILM (from 1.9 to 3.4%, $p = 0.01$).

To test the stasis hypothesis, the most comparable treatment modalities regarding lymph flow disruption are WLE vs. WLE/SLNB- and WLE/SLNB+/CLND vs. WLE/ELND. As metastasis already has occurred in WLE/DLND groups, this is not a good comparator. As ILM incidence according to meta-analysis doubled between WLE vs. WLE/SLNB- and increased almost threefold from 4.7% to 13.2% between WLE/ELND and WLE/SLNB+/CLND groups, ($p < 0.001$), the increase of ILM is unlikely to be due to the increase in lymph stasis. CLND and ELND are comparable in their amount of lymph flow disruption. This suggests that an aggressive tumor

behavior is the main reason for ILM, a statement that is supported by the spike in incidence after SLNB+, which is the patient group with the most aggressive tumor biology.

Limitations

Inevitable to any review, authors use different definitions and inclusion criteria. The level of heterogeneity is considerable, as illustrated in Table 1, where data on patient and tumor characteristics are shown. The inconsistent and varied application of terms as ITM, SL and LR complicate comparisons among trials. Recently some authors have even abandoned the concept of a true local recurrence, merging ITM, SL and local recurrence into locoregional metastasis, leading to considerable data loss.[57] Also, data on mitosis index, Breslow thickness and ulceration status were inconsistent, thus complicating comparisons, necessitating interpreting the results with caution. In general, patients included in SLNB studies have less favorable primary tumor characteristics than patients who undergo WLE alone.[58] Moreover, before introduction of the SLNB technique, patients with less favorable tumor characteristics were to undergo ELND and would therefore not be included in WLE studies. These limitations may account for the difference between this review and the MSLT-I, a prospective study, in which no increase in ILM or local metastasis was reported between biopsy and observation groups (7.7±1.0% and 8.4±1.3%, respectively; p = 0.38). As we included WLE patients before introduction of SLNB our WLE population would differ from the MSLT-I population.

The percentage of ILM after DLND in our study is lower than expected. This may be due to the small sample size and also due to bias as we only included ILM as FR after DLND. Since these patients have aggressive disease, they may more often progress to distant metastasis instead of locoregional disease.

Summary

This review showed an increase in ILM of 1.5% after only performing a SLNB procedure (ILM 1.9% for WLE vs. 3.4% for SLNB-). Taking into account the patient groups traditionally included in WLE studies it is difficult to say whether this increase represents an actual increase in ILM recurrence or a selection bias.

The SLNB procedure is the most important prognostic tool in clinical practice, providing a survival benefit in selected SLNB+ patients undergoing CLND and potentially serving as a marker to identify patients for adjuvant therapy. Sentinel lymph node biopsy has been suspected of causing to increase intralymphatic metastasis by restricting lymph flow. This review demonstrates this increase, but this result has to be interpreted with caution due to possible selection bias. As the stasis hypothesis seems to be incorrect based on the data in this study, aggressive tumor characteristics are likely the cause of this increase. We therefore advocate performing SLNB procedures, but to proceed with caution, adhere to the guidelines and not extend the indication area.

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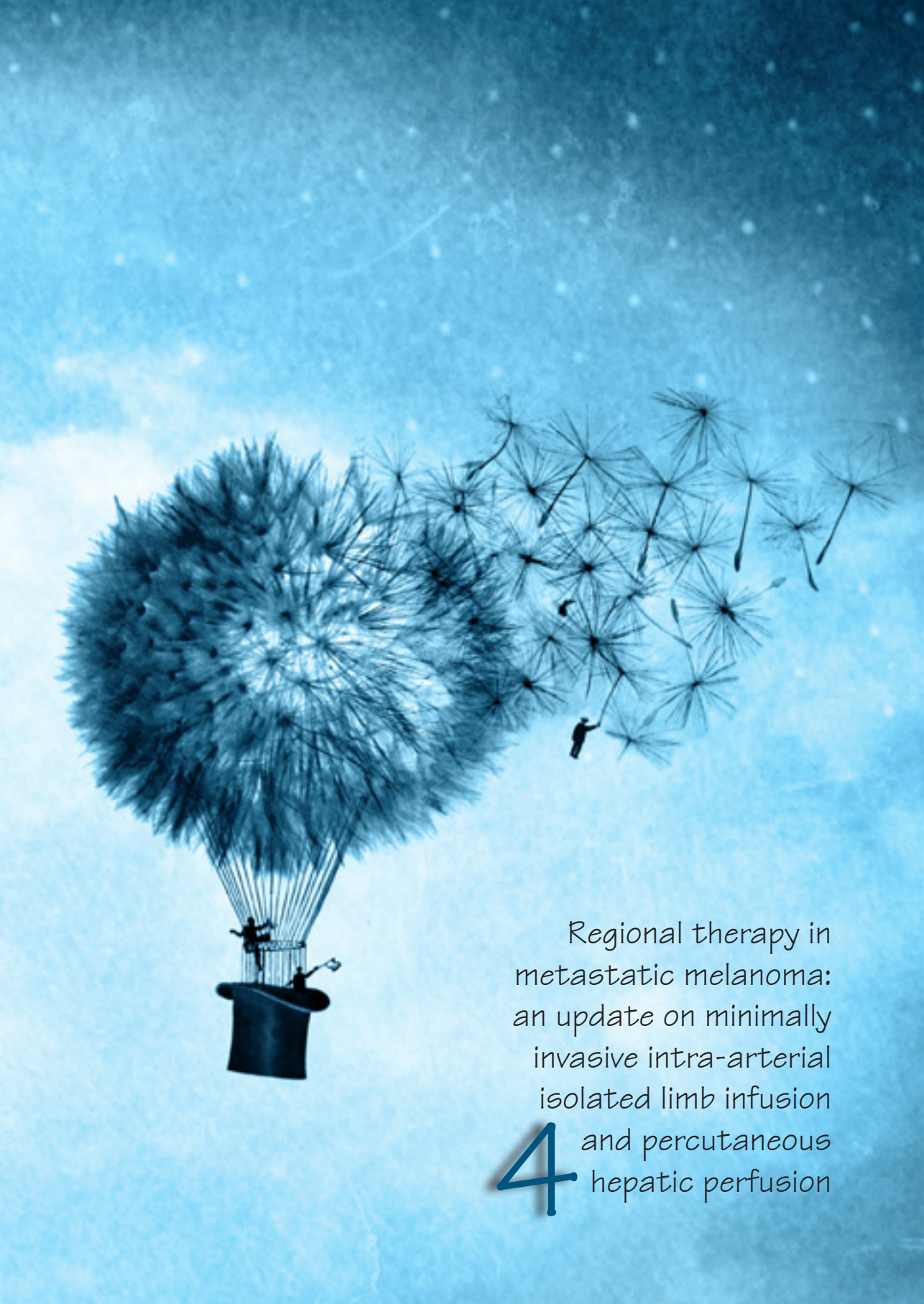
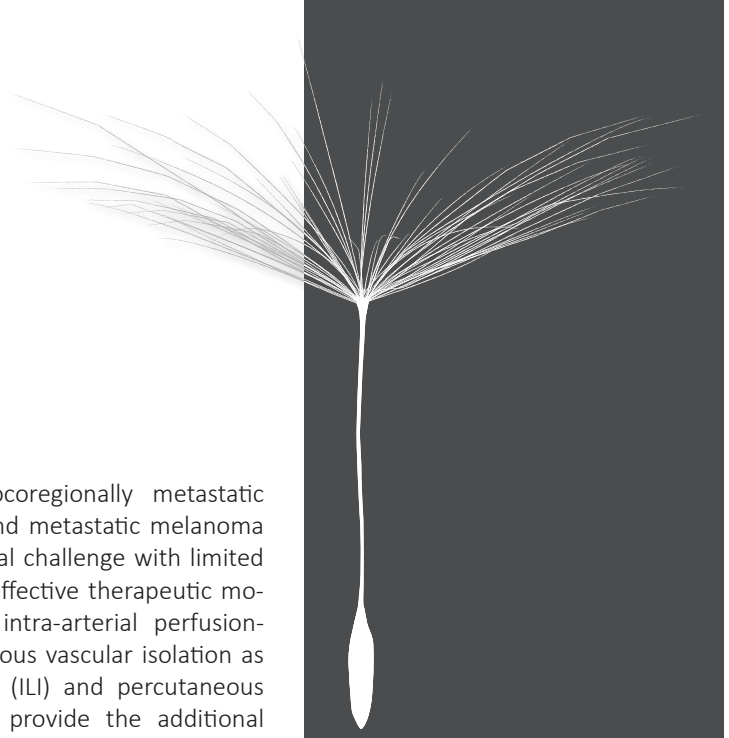


4 Regional therapy in metastatic melanoma: an update on minimally invasive intra-arterial isolated limb infusion and percutaneous hepatic perfusion



Abstract

Introduction

The management of locoregionally metastatic melanoma of the limb and metastatic melanoma to the liver poses a clinical challenge with limited therapeutic options. An effective therapeutic modality includes regional intra-arterial perfusion-based therapy. Percutaneous vascular isolation as in isolated limb infusion (ILI) and percutaneous hepatic perfusion (PHP) provide the additional advantage of minimally invasive techniques to further limit morbidity.

Areas covered

This review includes the technical aspects of ILI, PHP, the chemotherapeutic agents used and clinical responses. Also reviewed are pharmacokinetics and novel methods to enhance delivery of chemotherapeutics for both ILI and PHP and the efforts to improve therapeutic response and limit toxicity.

Expert opinion

Metastatic melanoma, particularly unresectable disease in the liver and in-transit disease in the limb, poses a clinical challenge with few effective treatments available. Although systemic therapy with immunotherapy or targeted therapy is an option, these modalities are associated with some systemic toxicity. Modalities that target treatment regionally, particularly minimally invasive techniques such as ILI and PHP, provide promising options to focus therapy on treating the affected limb or liver. The effectiveness of these minimally invasive methods has been supported by retrospective studies as well as prospective trials.

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Introduction

The goal of regional therapy is to deliver chemotherapy in high doses while simultaneously limiting systemic toxicity.[1-3] Historically, reports of regional therapy have included the extremity, liver, abdomen, pelvis, thorax, head and neck, and even the brain by surgically isolating the vessels to these sites to allow for intra-arterial therapy.[4-7] The vascular anatomy of the extremities and the liver are particularly amenable to isolation and intra-arterial regional perfusion-based therapy with much less morbidity than surgical isolation of the other mentioned anatomic sites.[4-7]

Although nearly half of all cases of cutaneous melanoma arise in the extremity, 2-10% of these cases may develop in-transit metastasis initially without distant disease.[8] In-transit metastasis is defined as tumor present in lymphatic channels which are found in the subcutaneous and dermal tissues.[8]

Control of in-transit disease does potentially offer a benefit for certain patients with metastatic melanoma because a subgroup will not develop distant metastasis despite a high burden of regional disease. The 5-year survival rates for patients with cutaneous in-transit and distant metastases range from 30 to 50% in patients with stage IIIB or IIIC disease, and <20% in patients with stage IV disease.[8-11] Accomplishing control of in-transit disease (stage IIIB/C) has demonstrated improved survival in select cases (25-30% 15-year survival).[8]

Although melanoma can present with diffuse metastatic disease, there are situations where even in the metastatic setting it will be limited to a single organ, such as the liver, as particularly seen in patients with uveal melanoma. Uveal melanoma is the most common primary malignant neoplasm of the eye with over half of these patients eventually developing distant metastasis. Interestingly, 80% of those patients who develop metastatic disease have the liver as the only site of metastatic spread.[8,9] Accordingly, it provides a scenario for liver-directed regional therapy in select cases. One-year survival for patients with uveal melanoma and liver metastasis is 10-15%, with <9 months median overall survival.[8-11] Furthermore, systemic therapy has demonstrated little efficacy, as trials utilizing chemotherapy produced only 5-20% response rates[8-11]. This review provides a comprehensive overview of all current data on isolated limb infusion (ILI) and percutaneous hepatic perfusion (PHP), including evidence on the pharmacokinetics (PK) of regional chemotherapy.

Isolated limb infusion

Method of drug delivery

The treatment of in-transit melanoma has focused on controlling disease at high risk of recurrence throughout the affected limb while preserving function. Intra-arterial regional therapy with chemotherapy (most commonly melphalan) treats all areas with disease and/or at high risk of disease in the affected limb without the morbidity of amputation.

To date, no other therapeutic modality has produced similar high rates of response for in-transit metastatic melanoma. Hyperthermic isolated limb perfusion (HILP) and ILI are proven therapeutic modalities shown to provide locoregional control of in-transit melanoma while preserving limb function.[8,12,13] In fact, reported durable complete response (CR) rates have ranged from 40 to 80% for HILP and from 30 to 38% for ILI.[8,12,13]

Regional therapies were first reported in the 1950s where open cannulation and surgical control of the vessels were achieved to provide intra-arterial regional chemotherapy.[4-7] Although HILP has demonstrated efficacy for in-transit metastatic melanoma, it is associated with morbidity of the open and complex surgery. With the evolution of percutaneous techniques and advancements in endovascular technology, minimally invasive techniques such as ILI have been introduced to accomplish regional intra-arterial chemotherapy without the morbidity of open and complex surgery and can be performed multiple times in the same patient.[8] The challenge to improve outcomes in these patients is not due to the technical aspects per se, rather it requires an optimization of delivering active agents delivered to cellular targets in the affected limb. Therefore, it is important to consider the PK efforts to improve response rates and limit systemic toxicity, as reviewed here (Table 1).

Intra-arterial administration of chemotherapy into the affected limb and vascular isolation are the hallmarks of HILP and ILI. The combination of an extremity tourniquet to prevent venous outflow and systemic toxicity with intra-arterial chemotherapy has demonstrated systemic leak rates of <1%, with the most serious systemic side effect being myelosuppression.[8,14,15] HILP requires open surgical control of the vessels with 12 French catheters, a 60 minutes circulation time, high flow rates (average of 400-600 cc/min), aerobic and oxygenated with a pump oxygenator and hyperthermia (41-41.5 °C).[8,14,15] ILI requires endovascular control with 6-8 French catheters in combination with a pneumatic tourniquet, 30 minutes circulation time, flow rates of 80-120 ml/min, hypoxia and acidosis and hyperthermia (37-39 °C).[8,14,15] Both techniques utilize washout at the end of the procedure.[8,14,15] Theoretically, the lower perfusion pressure in ILI may lead to less melphalan uptake by tumor cells than HILP, whereas the hypoxia and more profound acidosis in ILI may magnify the antitumor effects of melphalan compared to HILP.[8,14,15] Although the range of toxicities is similar between techniques, HILP has been reported to have a higher incidence of catastrophic toxicity requiring amputation (2.6 vs. 0%).[14]

Upper extremity ILI appears to be less morbid and potentially associated with a better response rate after repeat therapy than lower extremity ILI. In a retrospective study comparing 51 patients undergoing upper extremity ILI with 192 patients undergoing lower extremity ILI,

Table 1 - ILI studies

Author	n	Design	Findings
Beasley[12]	58 ILI vs. 54 HILP	Retrospective	ILI: 30% CR, 14% PR, 56% NR, 12 months CR, 18% toxicity, HILP: 57% CR, 31% PR, 12% NR, CR duration not reported, 32% toxicity
Kroon[13]	185 ILI	Retrospective	38% CR, 46% PR, 13 months duration of response, 22 months duration of CR, 53 months of survival, CR, stage of disease, primary melanoma, CO2 level and toxicity score correlated with outcome
Raymond[14]	62 HILP vs. 126 ILI	Retrospective	ILI: 43% CR + PR, 30% CR with 24 months duration, 28% CR after repeat treatment, 0% limb loss toxicity, HILP: 81% CR + PR, 55% CR with 32 months duration, 50% CR after repeat treatment, 3.2% limb loss toxicity
Chai[15]	44 repeat HILP or ILI, 70 ILI vs. 28 HILP	Retrospective	There was no statistical difference in survival or toxicity after the repeat procedures
Vohra[16]	22 STS ILI	Retrospective	42% overall response rate (24% CR, 18% PR, 18% SD, 41% PD), unknown duration of response
Wong[17]	77 LE ILI vs. 27 UE ILI	Retrospective	Improved ORR in repeat UE ILI than LE ILI, longer length of stay and toxicity in LE ILI
Beasley[21]	19 TMZ ILI at MTD	Phase 1 dose escalation	10.5% CR, 5.3% PR, 15.8% SD, 68.4% PD without dose-limiting toxicities at the MTD
Turaga[22]	22 non-melanoma ILI	Retrospective	79% ORR, 21% CR, 58% PR, 4% grade 4 toxicity, unknown duration of response
McMahon[29]	13 ILI vs. 29 ILI corrected for IBW	Observational study	No statistical difference in response rate, but lower toxicity in the corrected group including compartment syndrome
Beasley[39]	51 UE ILI vs 192 LE ILI	Retrospective	UE ILI had lower limb volumes, melphalan doses, lower ischemic times, toxicity, but no difference in CR compared to LE ILI

CR: complete response; HILP: hyperthermic isolated limb perfusion; IBW: ideal body weight; ILI: isolated limb infusion; LE: lower extremity; MTD: maximum tolerated dose; NR: no response; ORR: overall response rate; PD: progression of disease; PR: partial response; SD: stable disease; STS: soft tissue sarcoma; TMZ: temozolomide; UE: upper extremity.

there was a lower rate of toxicity in the upper extremity group, without any difference in the response rate.[16]

In addition, in another study where patients underwent repeat ILI, those patients who had upper extremity ILI had better overall response rates than those with lower extremity ILI.[17] Reported CR rates have been 40-80% and 30-38% for HILP and ILI, respectively.[8,12,13] Although HILP has been considered the standard treatment, ILI provides a less morbid alternative, with HILP reserved for cases that fail initial ILI therapy or cases with positive lymph node disease.[8]

Regional therapy with melphalan

Melphalan is an alkylating agent known as L-phenylalanine mustard that has been in use for regional therapy since the earliest reports in the 1950s.[4-7] The mechanism of action focuses on both resting and dividing cancer cells.[8] Although melphalan has not been demonstrated to have efficacy against metastatic melanoma when administered systemically, regional intra-arterial high dose administration has demonstrated efficacy.[8,18] The difference has been thought to be secondary to the 10- to 100-fold higher maximally tolerated doses that are achieved in regional versus systemic administration.[8,18]

Although melphalan is widely accepted as the standard agent for regional perfusion therapy, there are some interesting data in animal models that temozolomide may improve response rates.[19] Temozolomide is an imidazotetrazine derivative of dacarbazine, an alkylating agent, and it rapidly converts to an active 3-methyl-(triazen-1-yl)imidazole-4-carboxamide compound which interferes with DNA replication.[19] Although the efficacy of this agent systemically has not been demonstrated to be any better than dacarbazine, there is an interest in its role in high-dose ILI circuits.[19,20] A phase 1 dose escalation trial of temozolomide-ILI PK study in 28 patients demonstrated minimal toxicity.[21]

Though melphalan has been used for regional perfusions performed for metastatic melanoma, there are some interesting data to consider it for the treatment of other cutaneous and soft tissue malignancies that present with locoregional metastatic disease in the limb. Response rates for non-melanoma cutaneous malignancies and soft tissue sarcoma in small series of patients have ranged anywhere from 42 to 79%.[16,22] However, the duration of response is unknown, as the retrospective studies did not have data on long-term follow-up.[16,22]

In regional administration, the peak level of melphalan-induced DNA interstrand crosslinks is achieved four hours after infusion, followed by a gradual decline. Cellular uptake of melphalan achieved saturation after ten minutes as demonstrated by in vitro studies.[8] PK studies using animal HILP models demonstrated rapid uptake of melphalan with a linear dose-response relationship to toxicity, similar to HILP studies in humans. Because of these features, melphalan is considered the drug of choice for both HILP and ILI.[8,23]

Appropriate melphalan dosing balances maximal response against systemic toxicity. Historically, this calculation has been based on total body weight; however, studies have demonstrated that such calculations result in doubling the dose between two different patients with the same size extremity.[8] Because of the broad spectrum in body habitus and the variable distribution of fat, muscle and other tissues between different patients, total body weight is not an accurate metric.[8,24] Instead, limb volumetric measurements based on water displacement or circumferential measurements of the portions of the limb to be treated provide greater accuracy for dosing calculations.[8,24] The most commonly used doses of melphalan for the upper and lower extremities are 13 and 10 mg/L for HILP and 10 and 7.5 mg/L for ILI, respectively.[8,12,24] To balance against the toxicity produced from peak perfusate concentrations, melphalan is infused over 5 minutes for a 60 minute perfusion time in HILP, and over 2-5 minutes for 30 minutes of circulation time in ILI.[8,12,24] In a study of 171 patients, adjusting for ideal body weight (IBW) did not affect therapeutic response, but it did significantly reduce toxicity.[25]

Tumor drug delivery

The limitations of assessing tumor drug delivery have been due to PK studies relying on plasma drug concentration, which varies in different tissues where tumor may be found.[8] Consequently, plasma concentration of melphalan does not necessarily correlate with its concentration in a tumor, tumor response or even extremity toxicity.[8,26] In contrast, microdialysis measures melphalan concentration in these sites, which has demonstrated a correlation between tumor response and melphalan subcutaneous microdialysate concentration without any correlation to extremity toxicity in ILI.[8,26] Further investigations are underway utilizing microdialysis to assess toxicity. In addition, functional imaging, such as MRI, has been applied to assess the effects of melphalan on tumor micro-environment. While studies are still underway, potential applications in the future include an assessment of the kinetics of contrast perfusion as a correlation to drug delivery and perhaps even therapeutic response.[8,26]

Models for predicting outcomes

The model for understanding and studying the PK of intra-arterial chemotherapy in the limb is based on a two-compartment system.[8,27] When the drug is administered into the limb, it is first distributed into the central compartment, followed by distribution into the peripheral compartment. Although compartments in the extremity are traditionally thought of along anatomic boundaries, this model instead categorizes tissues by how quickly they are perfused by the drug. That is, tissues that are perfused quickly are in the central compartment, whereas those that require more time to be perfused are in the peripheral compartment. Applying this model, the plasma melphalan concentration over time can be fitted to a biexponential equation (WinNonlin Version 2.1, Scientific Consulting, Inc.), which agreed with actually measured values in HILP and ILI.[28]

Application of the two-compartment model in a study of 14 patients undergoing HILP for melanoma demonstrated differences up to fivefold in melphalan concentrations using the same dosing guidelines discussed above.[8,28] The ratio of estimated limb volume to steady-state limb drug volume of distribution (Vesti:Vss) directly correlated with toxicity. Patients with a high Vesti:Vss were more likely to have actual body weight (ABW) greater than IBW.

In fact, when the melphalan dose was modified by a ratio of the IBW:ABW, there was a reduction in high-grade toxicity (15 vs. 50%) without a change in the CR rate. These results were supported by further studies.[8,25,28,29]

Currently, PK modeling has failed to consistently predict toxicity and clinical response to therapy - key components of the decision-making process to proceed with treatment. In fact, as many as 20% of patients present with toxicities unexplained by current models and a large percentage of patients fail to have a CR or durable response to therapy.[8] Understanding the limitations of these models will help guide efforts to better predict risks and benefits of HILP and ILI.

Because animal models demonstrate a plateau of therapeutic response independent of drug concentration, it is thought that tumor biology limits response despite any goal concentration of drug achieved.[27] For this reason, inquiry has focused on the reported mechanisms of melphalan resistance: downregulation of cellular transmembrane transporters, intracellular drug inactivation, DNA crosslinking repair and drug efflux.[8,30] Accordingly, there are studies underway to address biological mechanisms for the discrepancy between predicted and actual response to therapy. However, currently there are limits to the translation of these findings to the clinical setting. For example, although hyperthermia has been demonstrated to increase drug uptake in vitro, in vivo models have not supported increased drug uptake as the mechanism for the enhanced melphalan cytotoxicity in hyperthermia.[31,32]

Because the in vivo system introduces microenvironment, blood flow and other factors of greater complexity than can be reproduced in a basic in vitro system, there are investigations to understand the implications on PK to improve models for predicting outcomes. For example, it has been shown that patients who demonstrate a partial response (PR) are more likely to have an increased disease-free survival if lesions are resected after ILI when compared to cases that do not demonstrate a PR.[17] In addition, patients with a lower overall tumor burden demonstrate improved overall response rates to ILI than those with a higher tumor burden in the affected limb.[33] Such findings are not explained by PK alone and implicate the importance of considering other potential targets for combined therapy.

Role of targeted therapy

As noted above, metastatic melanoma interacts with its surrounding microenvironment to develop aberrant blood supply, independent of the supply of normal surrounding tissue.[8] Although this difference in blood flow is most notably exploited in the approach to liver metastasis, there have been efforts in the limb to molecularly target this difference to further improve outcomes in ILI. ADH-1 induces disruption of N-cadherin complexes resulting in increased vascular permeability.[8] As a target to improve drug delivery in combination with melphalan via ILI, animal studies demonstrated decreased tumor growth and increased apoptosis compared with ILI alone.[34,35] A phase 2 trial in 42 patients demonstrated that the combination of ADH-1 and ILI was well tolerated with a 16% additively increased tumor response rate and an increase in N-cadherin measured in tumors but without any difference in overall time to in field progression of disease.[29]

These findings may support the argument that improving drug delivery alone may not be sufficient to improve the completeness and duration of response to therapy.[8] A target of angiogenesis that has attracted a great deal of interest is the VEGF, which has been implicated in all aspects of vascular development, growth and permeability, in physiological and pathological states including metastatic melanoma.[8] In fact, targeted therapy utilizing the mAb to VEGF (bevacizumab) has been approved by FDA for therapy in colorectal, brain and lung cancers.[8] Of the potential applications for intra-arterial chemotherapy, studies have demonstrated that bevacizumab results in changes in the tumor blood supply, similar to that found in the surrounding normal microvasculature.[8,36] This may offer a benefit in the case of regional intra-arterial therapy to increase blood flow to the in-transit lesions in the limb, as the catheters are infused via the main artery and thus make use of normal channels of blood flow to the tissues in the affected extremity. This normalization of the microvasculature with increased melphalan tumor delivery and tumor response caused by bevacizumab was demonstrated in animal models.[37] However, clinical data are needed to further study the role of this pathway in this treatment modality.[8]

Other important classes of targets for therapy in melanoma are linked Raf serine/threonine kinases, receptor tyrosine kinases and the RAF-MEK-MAPK signaling pathway, which are associated with both cancer proliferation and survival in metastatic melanoma.[38] Sorafenib is a multikinase inhibitor that blocks these pathways and has been found to inhibit the activity of VEGF receptor (VEGFR) tyrosine kinase.[8] Similar to the discussion above on the potential applications to enhance drug delivery in regional therapy via targeting the tumor microvasculature, results from an animal model demonstrated slow tumor growth when sorafenib was combined with ILI.[34] However, a phase 1 trial of sorafenib and melphalan-based ILI combination in 20 patients resulted in increased toxicity without seeing an appreciable increase in clinical response.[39] Although patients treated with sorafenib have been reported to have reduced VEGFR2 expression, a factor reported to potentially correlate with clinical response, inhibition of the RAF-MEK-MAPK pathway has not been demonstrated in sorafenib-treated tumors.[8] There appears to be a correlation between the dose of the sorafenib and the degree of VEGFR expression (lower in 600 than 400 mg/day).[40]

With the proliferation of agents targeting immunotherapy in melanoma in the past few years, there has been an interest in exploring the role of such agents as ipilimumab, the CTLA-4 mAb. In an animal model, ipilimumab alone versus ILI/ipilimumab demonstrated no increased response, but there was an increase in CD8 cells as well as antigen-specific tumor cell infiltration.[41] Clinical data are needed to understand the role of immunotherapy in ILI.

Percutaneous hepatic perfusion

In patients with hepatic metastases, complete surgical resection offers the best improvement in overall survival and is the only potentially curative option.[42] However, only a small minority

of patients (2-9%) classify as a surgical candidate.[42,43] Therefore, when there is no extrahepatic disease, regional intra-arterial therapies that deliver high doses of chemotherapeutic agents to tumor cells locally are the preferred method of treatment, thereby minimizing systemic side effects.[1-3] Even large tumors, covering >50% of the liver, can be treated this way.[42]Neoadjuvant downstaging and two-stage hepatectomies may increase the number of resectable tumors in select patients. Uveal melanoma metastatic to the liver, which is usually not amenable to surgical resection, poses a unique challenge. Despite recent successes in metastasized cutaneous melanoma and investigation of a wide range of agents, in uveal melanoma, none have shown sufficient activity to progress to a phase 3 trial.[42,43] The modalities available to the locoregional treatment of unresectable metastatic cancer to the liver include ablative techniques, radiotherapy and chemoembolization. However, the effectiveness of ablation and embolization techniques is limited by the number and size of liver metastases, and radiotherapy and chemoembolization have not proven to have an impact on survival. Because liver metastases derive the majority of their blood supply from the hepatic artery, the same principles of regional therapy have been applied to this clinical scenario in isolated hepatic perfusion (IHP) and PHP to deliver chemotherapy via the hepatic artery circulation.[44,45] IHP has demonstrated promising results, as have recent data on PHP in select patients with metastatic melanoma isolated to the liver.[1]

Technique

The first treatment described for isolated treatment of liver metastasis was IHP in 1961.[46] Although effective, IHP is a major operative procedure that requires a laparotomy with a duration of 8-9 hours and a prolonged hospital stay, leading to considerable morbidity and mortality. Beheshti et al. therefore developed a minimally invasive percutaneous technique in the early 1990s without the morbidity of a laparotomy.[44] This technique was further refined by Alexander, Bartlett, Pingpank et al. at the National Cancer Institute.[47-49]

PHP is based on the principle of treating liver metastasis with a high-dose chemotherapeutic agent, while limiting systemic toxicity, by taking advantage of the unique aspects of arterial inflow and venous outflow of the liver. PHP is especially important in uveal melanoma, where 95% of patients who develop metastatic disease will have liver metastases, which in 80% of cases will be the only site of distant disease. PHP has also successfully been described in metastasized colorectal cancer, sarcoma, hepatocellular carcinoma and cutaneous melanoma.[1,50].

Liver metastases obtain inflow primarily from the hepatic artery, as opposed to normal hepatocytes that derive 50% of their blood flow from the portal venous system. High doses of chemotherapeutic agents, thus, can be infused into the hepatic artery and directed right at the tumor.[44-46] Hepatic arterial infusion provides the additional advantage of a 10-fold higher intratumoral concentration of chemotherapy when compared to portal vein infusion.[51] Because venous outflow into the systemic circulation for the entire liver is via the hepatic veins into the inferior vena cava (IVC), vascular isolation of the liver can be achieved via balloon occlusion of the IVC. This also allows for filtration of the chemotherapeutic agent with a veno-venous bypass before it reaches systemic circulation in order to limit systemic toxicity.

The procedure starts by inserting the inflow catheter into the hepatic artery and embolization of any accessory arteries to prevent infusion of melphalan into any other organs outside of the liver. Vascular isolation of the liver is achieved by inserting a double-balloon catheter system (Delcath, Inc., NY, USA) into the IVC. The distal and proximal balloons are positioned superior and inferior to the hepatic veins. Balloon inflation under fluoroscopic guidance occludes the IVC. The catheter is then attached to an extracorporeal circuit. The venous outflow is circulated into the pump and subsequently into two parallel connected proprietary filtration cartridges, thus creating a veno-venous bypass with in line hemofiltration. The filtered blood is returned to the systemic circulation veins via an introducer catheter placed in the internal jugular vein.[8,50]

Because of these features, PHP provides several advantages over IHP, which include the ability to repeat treatments in the same patient with reduced toxicity and morbidity. In fact, as many as six procedures have been described in a single patient, with a median hospital stay of three days.[2,50,51]

Outcomes in melanoma

Four trials investigating the use of PHP have been conducted in the past 20 years, which demonstrated promising results (Table 2). Two phase 1 trials have been published by Ravikumar et al. and Pingpank et al. The series by Ravikumar et al. included 23 patients with various liver tumors treating 21 of them, out of which two were melanoma patients, with either doxorubicin or 5-fluorouracil.[52] Two patients, one of whom was a melanoma patient, achieved a PR. This patient experienced a 50% reduction in the liver metastases after two PHP treatments with doxorubicin and a 96% reduction after four treatments. Treatment details for the second melanoma patient were not reported.

Pingpank et al. performed 74 PHP treatments with melphalan on 28 patients every 4-8 weeks.[48] A total of 27 patients were available for evaluation, among whom ten were uveal melanoma patients and two were cutaneous melanoma patients. Response was seen in 50% of the uveal melanoma patients (3 PR, 2 CR) but not in cutaneous melanoma patients. Based on these data, Pingpank et al. completed the first, and only, phase 3 trial comparing melphalan PHP to best alternative care (BAC) in 93 patients with uveal and cutaneous melanomas.[47] Up to six PHPs at four to eight week intervals were given, provided the patients did not show disease progression. Patients in the BAC group were permitted to crossover to PHP on hepatic progression. Median hepatic progression-free survival was 245 days in the PHP group versus 49 days in BAC ($p < 0.001$) group. Overall response rate was 34 and 2%, respectively, indicating a benefit from PHP ($p < 0.001$). The study showed no benefit in overall survival, which may be due to the crossover design, as 28 BAC patients crossed over to PHP and 27 of these received PHP. For BAC, PHP and BAC-PHP crossover, median overall survival was 9.8, 4.1 and 15.3 months, respectively.[53]

Table 2 - PHP studies

Author	n	Treatments	No. of treatments	Perfusion time (min)	Drug	Response	hPFS	OS (months)
Ravikumar[52]	2	PHP	2 (median)	15 - 30	Fluorouracil Doxorubicin	50% PR	NR	NR
Pingpank[48]	12	PHP	2.5 (mean)	30	Melphalan	42% ORR	NR	NR
Pingpank[47,53]	93	PHP (n = 44)	3 (median)	30	Melphalan	34% ORR	8.0 months	9.8
		BAC (n = 49)*			BAC	2% ORR	1.6 months	4.1
		[Crossover n = 28]*			BAC/melphalan	n/a	8.8 months	15.3
Forster[50]	10	PHP	3 (median)	30	Melphalan	56% PR	240 days	NR

**Patients on BAC were allowed to crossover to PHP after hepatic progression.*
BAC: best alternative care; hPFS: hepatic progression free survival; n/a: not reported; ORR: objective response rate; OS: overall survival; PHP: percutaneous hepatic perfusion; PR: partial response.

A single institutional experiment at Moffitt Cancer Center by Forster et al. reported results of PHP using melphalan in ten patients with hepatic metastasis of cutaneous melanoma (n = 3), uveal melanoma (n = 5), melanoma of unknown primary (n = 1) and leiomyosarcoma (n = 1).[50] Six of the patients were treatment naive. Patients underwent a median of three PHP treatments (range 1-4). There was a 90% disease control rate with four patients having stable disease and 5 having a PR. Only one patient with uveal melanoma progressed on initial post-procedure restaging imaging. One patient with cutaneous melanoma in the liver has not progressed after 1337 days. The median hepatic progression-free survival was 240 days after a median follow-up of 11.5 months. At the time of publication, the median overall survival was 12.6 months from the time of diagnosis of hepatic metastases.

Pharmacokinetics

Although in the early phase 1 trials both melphalan and doxorubicin were used for PHP, melphalan is the agent of choice after a dose escalation study by Pingpank et al.[47,48] It is a very suitable chemotherapeutic agent because of its high first pass metabolism, high hepatic clearance rate, dose-dependent toxicity and enhancement of its effects by hyperthermia.[52] Locoregional melphalan levels decline steadily during perfusion, indicating a rapid uptake by liver tissue, with most of it cleared within 10 minutes after infusion.[48,54] In PHP, isolation of the hepatic perfusion system has been shown to increase locoregional concentrations of melphalan up to 10-fold as compared to intravenous administration in an animal model. Further, during perfusion, drug concentrations in the liver are 20- to 40-fold higher than systemic concentrations, thus showing a low systemic exposure to the drug.[8] The mean filter rate extraction during the procedure is 77%. Thus, melphalan PHP provided high treatment doses to the disease in the liver with reduced systemic exposure, limiting toxicity. Melphalan is used at a dose of 3 mg/kg based on IBW, as determined in a phase 1 dose escalation study by Pingpank et al.[48]

Complications and toxicity

Although PHP has a lower risk profile than IHP, it is not without its own risks and complications. However, dose-limiting adverse events are rare. Complications can be categorized as those related to percutaneous catheterization, hepatic isolation with veno-venous bypass along with the drugs administered such as heparin and protamine sulfate and melphalan infusion. Due to the small number of patients currently reported in the literature and the fact that not all studies present a comprehensive summary of adverse events, the frequency of these events has to be interpreted with caution. Ravikumar et al. present the most comprehensive summary of side effects but did not use the current drug of choice, melphalan, in their study.[52] Percutaneous catheter placement associated complications include hepatic artery dissection, pneumothorax and hematoma at the balloon insertion site.[52] Hepatic isolation associated complications include hypoxemia, hypothermia, hemodynamic instability, heparin-induced thrombocytopenia, mild elevations in troponin and hepatic enzyme levels, protamine reactions and deep venous thrombosis.[51] Transient metabolic acidosis during the procedure is

very common, as is hypotension after balloon inflation (78.5%), caused by fluid administration prior to balloon inflation and the return of hepatic venous blood via the internal jugular or subclavian veins. Hypotension also occurs after flow is diverted through the filters, as the filters remove endogenous catecholamines on top of chemotherapeutic agents, and by hemodilution caused by the veno-venous bypass system.[51] Hypotension is usually transient and is treated with pressors such as norepinephrine and vasopressin by anesthesia. The infusion of melphalan is not begun until the mean arterial pressure is >70 mmHg and the hepatic arteries demonstrate no spasm on repeat angiogram. Spasm of the hepatic arteries can cause retrograde flow into the stomach and/or duodenum and potentially cause damage to these organs due to high concentration of melphalan directed into their feeding vessels. Spasm is relieved by nitroglycerin into the hepatic artery. The process of the PHP allows for rechecking of hepatic artery spasm after each 100 cc aliquot of chemotherapy (at 25 cc/min) is given, therefore identifying and correcting spasm if it may occur before higher doses are diverted to the stomach or duodenum. As a prophylaxis, the gastroduodenal artery and any branches off the gastric or hepatic arteries are embolized pre-perfusion to make sure that there is no collateral flow to unwanted area.

Melphalan-induced associated complications include myelosuppression and systemic toxicities due to systemic leak to surrounding organs (e.g., gastritis).[8,48,52,53] In the phase 1 trial, at the currently used dosage of melphalan (3.0 mg/kg), grade III/IV neutropenia, thrombocytopenia and anemia were noted in 73.7, 36.8, and 21.1%, respectively.[48] Postoperatively, nausea and vomiting are common (10%).[52] It is expected that future improvements in chemofiltration will reduce bone marrow suppression and other manifestations of systemic leak. As of the publication date of this article, the FDA in the USA has not approved the PHP for use. It is currently being used on an expanded access protocol as well as compassionate use cases. The device is available for commercial use in the EU (CE Mark approved). Further phase 2 and phase 3 protocols are being planned.

Conclusion

Unresectable melanoma to the liver and unresectable in-transit disease in the limb pose a clinical challenge with limited options for treatment; however, ILI and PHP provide the opportunity to treat with high-dose chemotherapy utilizing minimally invasive techniques with minimal morbidity and is associated with minimal systemic toxicity. Further, these regional therapies have demonstrated improved response rates when compared to the results of standard systemic therapy in select patients.

Expert opinion

When the metastatic melanoma is limited to the limb or liver, regional therapy is an important option to consider, especially the minimally invasive ILI and PHP techniques. The benefits they provide include a percutaneous approach that avoids the morbidity of open and complex surgical procedures, the ability to perform multiple treatments as well as use other agents in the setting of clinical trials. Further, ILI and PHP have demonstrated efficacy in achieving control of disease confined to the limb or liver. These two features are important to consider in a patient population whose goals of therapy are to control their disease which oftentimes is symptomatic and to develop new treatment options to improve disease-specific survival. In addition, because control of locoregional disease in select cases has demonstrated durable improvements in survival, further studies to guide patient selection offer the potential to further improve outcomes.

ILI and PHP also provide a unique opportunity to evaluate novel therapeutic agents without any added risk to the procedure. Readily available tumor for biopsy in the field of an ILI as well as a closed circuit makes in ILI and PHP attractive procedures to perform real-time tumor biopsy to assess treatment affect and PK studies on the effluent with the chemotherapy in the closed circuit. Such features are important to consider because further studies are necessary to predict which patients will benefit from these interventions to improve outcomes. In vivo systems introduce microenvironment, blood flow and other factors of greater complexity for investigation, factors which cannot be reproduced in a basic in vitro system. Even assessing tumor drug delivery itself has been limited by reliance on plasma drug concentration, for the actual concentration of drug delivered varies in different tissues within the limb or liver perfused. Because of this variable distribution, plasma concentration has not reliably predicted response or toxicity. Although preliminary findings in the role of targeted therapy, especially in the exciting era of immunotherapy, are promising, further development of these models for understanding and predicting response to regional therapy will be critical for translating such preliminary findings into clinical application.

The question remains what applicability these animal models truly have in predicting outcome. Although they do offer advantages over the in vitro systems by providing a more complex system for experimentation, their limitations are important to consider, especially in regional therapy. ILI and PHP rely on human patients responding to a treatment by immunologically destroying human cancer cells. This system may be too complex to reliably test in animal models and may explain some of the discrepancies between the results of animal studies and preliminary clinical data. These challenges underscore the importance of well-designed clinical trials that assess outcomes and address biological mechanism.

Because of the easily accessible bypass circuit, real-time PK data can be readily obtained. ILI has the added feature of providing access to tumor tissue in the treated field during the entire time course of treatment via tumor biopsy of subcutaneous lesions with minimal morbidity. Although PHP may not offer access to tumor tissue as in ILI, the imaging modalities available to assess the tumors, including angiography during the procedure itself, do offer areas for

investigation to address the questions of tumor blood flow. Utilizing these features in clinical trials may be of particular applicability in assessing the role of targeted therapies, especially in the field of angiogenesis. Therefore, clinical trials addressing the factors discussed above offer the opportunity to understand how these factors impact treatment response and toxicity with tremendous implications on PK to improve models for predicting outcomes. Further, with the rapid proliferation of novel agents for systemic therapy in melanoma over the past few years, the potential to combine these novel systemic and regional therapies in prospective trials is critical for patients with advanced melanoma.

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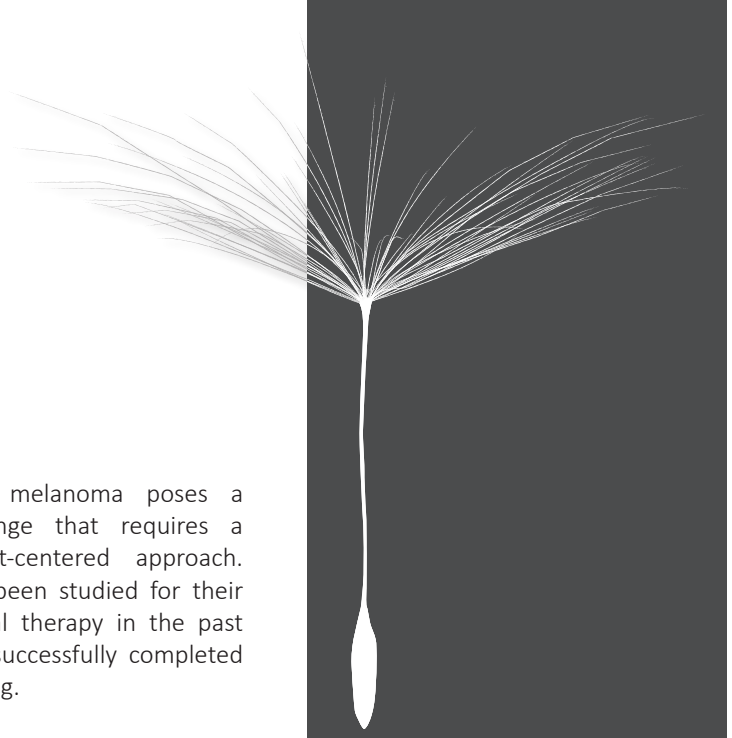
Developments in intralesional therapy for metastatic melanoma



Abstract

Background
Locoregional advanced melanoma poses a complex clinical challenge that requires a multidisciplinary, patient-centered approach. Numerous agents have been studied for their suitability as intralesional therapy in the past decades, but few have successfully completed phase 3 clinical trial testing.

Methods
The relevant medical literature was searched for articles regarding use of intralesional therapies in metastatic melanoma. Therapies with data from phase 2 or higher studies were selected for review. This review also summarizes the mechanisms of action, adverse event profiles, and clinical data for these agents.

Results
Intralesional therapies demonstrate promising effects in select patients with advanced melanoma. The optimal approach should be individually tailored and consist of a combination of intralesional therapies, regional perfusions, systemic immunotherapies, targeted therapies, and surgery, if necessary.

Conclusions
Due to its relatively good local response rates and tolerable adverse event profile, intralesional therapy may be a treatment option for select patients with unresectable locally advanced or metastatic melanoma.

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Introduction

Melanoma is accountable for most deaths related to skin cancer.[1] In 2016, an estimated 76,380 new cases of melanoma will be diagnosed and approximately 10,130 people will die from the disease in the United States alone.[1] Although cure rates are high if the disease is discovered when confined to its primary location, metastasis frequently occurs.[1] An unique clinical challenge posed by locoregional metastasis, also known as intralymphatic metastasis, occurs when metastasis develops between the primary melanoma and the draining lymph node basin. This type of metastasis, which occurs in 5% to 10% of patients with melanoma, has traditionally been classified into two categories: satellite metastasis (located <2 cm from the primary tumor) and in-transit metastasis (located ≥2 cm from the primary tumor).[2,3]
Surgical resection is the standard of care for patients whose disease is limited enough to be rendered with no evidence of disease. If disease is confined to the limb, then unresectable disease can be amenable to locoregional treatment. For example, regional perfusion therapies, such as isolated limb infusion or hyperthermic isolated limb perfusion, have demonstrated objective response rates (ORRs) of 50% to 90%.[4,5] These treatments can be repeated multiple times, depending on response and rate of toxicity. The disadvantages of limb infusion and perfusion include associated regional toxicity, morbidity from a surgical procedure, and applicability to disease confined to the extremities alone (e.g. not applicable to in-transit metastasis on the trunk). Although radiotherapy is frequently used to treat microscopic disease in an adjuvant setting and has been used to treat individual lesions or localized clusters with anecdotal success, macroscopic melanoma is difficult to treat with radiotherapy; however, wide-field irradiation is associated with morbidity and is not a preferred first-line modality.[6,7]
Patients with limited locoregional disease often have few symptoms. Consequently, physicians are less likely to recommend systemic or regional perfusion-based therapy that could expose asymptomatic patients to considerable toxicity. These patients may benefit from intralesional therapy, where the active agent is immediately injected into the tumor, exerting mainly local effects, with fewer adverse events than systemic or regional therapy.[3] Intralesional therapies have been extensively studied, but effective agents have not been available until recently.[6] However, similar to the rapid development of multiple new systemic treatments for stage III/IV metastatic melanoma (nivolumab, ipilimumab, trametinib, dabrafenib, vemurafenib, pembrolizumab, cobimetinib, pegylated interferon), intralesional injections and topical therapies have seen major advances.[8,9] Due to their rate of efficacy and relatively low toxicity profile, these treatment modalities may be promising in select patients with locoregional disease.[3]

Intralesional therapy was first reported in 1893 by Coley,[10] which was prior to the report published by Handley[11] on wide local excision as the mainstay of melanoma treatment. Local therapy increases rates of efficacy and lowers rates of toxicity when compared with systemic administration by delivering an increased concentration of the drug locally.[3,12] A so-called 'bystander effect' has been reported in select agents, including velimogene aliplasmid, 10% rose bengal, and talimogene laherparepvec, where noninjected (both visceral and nonvisceral) distant lesions respond to the locally injected drug.[9,13] Although the exact mechanism of action is under investigation, tumor antigens in the injected lesions may serve as an autologous vaccine, stimulating systemic immunity.[12,14] The occurrence of the bystander effect makes intralesional therapies appealing because local injections have been associated with a systemic reduction in tumor burden.[15]

Generally, lesions are treated using a 25- to 30-gauge needle using a 'fanning' technique, where the needle is moved in multiple directions within the same lesion. Preferably, the same needle entry and needle stick are used to keep the number of needle tracks and cavities in the tumor limited to prevent intralesional injectate from leaking out and to maximize the delivered dose. Visible or palpable lesions can be injected in the ambulatory clinic, whereas deeper lesions can be injected using ultrasonographic guidance. Tumor response may be measured using caliper measurements, ultrasonography, or cross-sectional imaging (magnetic resonance imaging/computed tomography), depending on tumor size and location.[9] Evidence suggests that subcutaneous lesions are less responsive than cutaneous lesions, and tumors with smaller bulk are more likely to regress under treatment.[16-18] Investigators have attempted to limit intralesional volumes to 1 mL or less to minimize the local adverse events that result from injecting higher volumes.[16]

This review will summarize the mechanisms of action, adverse event profiles and clinical data for all agents currently in use and of historic importance (Tables 1 and 2).[9,12,13,16,18-41]

Velimogene Aliplasmid

Velimogene Aliplasmid is an intralesional agent that advanced to phase 3 clinical trial testing based on results seen in phase 1/2 trials; however, both phase 3 trials conducted with velimogene aliplasmid failed to reach their primary endpoint (NCT00395070).[24,25] Velimogene aliplasmid is classified as a gene therapy because it contains plasmid DNA encoding for HLA-B7.[25] It recruits macrophages and T cells, which attack injected and noninjected lesions alike, bringing about immune responses against the alloantigen. Most of the initial studies were limited to study participants negative for HLA-B7; however, after no correlation between HLA status and response rate was found, other studies did not incorporate HLA status as an inclusion criterion.[16] Reported adverse events include paresthesias, asthenia, myalgias, fatigue, injection-site pain, rigors, and flulike symptoms.[16]

Velimogene aliplasmid was first investigated in four small phase 1 trials with up to 17 study participants and reported response rates reaching 50%.[20-23] The study of this drug advanced to four phase 2 trials that reported ORRs ranging from 10% to 28%.[16,25,27] The most frequently reported schemes used 2 mg velimogene aliplasmid per lesion with 1- to 2-week intervals.[16,27]

The largest study was a dose escalation/efficacy trial conducted by Bedikian et al.,[16] who enrolled 133 patients and assigned them to groups that received 0.5 to 2 mg velimogene aliplasmid for six weeks with one week intervals. A total of 127 participants were treated with the highest dose; efficacy data were also available for all enrollees.[16] Complete response (CR) was reached in 3% and partial response (PR) in 9%.[16]

In the first phase 3 study, Richards et al.[24] randomized 202 patients to either systemic dacarbazine/velimogene aliplasmid on days 3 and 10 out of 28 of the chemotherapeutic cycle (n = 98) or dacarbazine alone (n = 104). Response rates were 13.2% and 11.6%, respectively.[24] Adding velimogene aliplasmid did not cause any significant difference in median time to progression (1.9 vs. 1.6 months) or survival (10.8 vs. 9.2 months).[24] The second phase 3 trial was stopped early when no difference was shown in ORR at more than 24 weeks and in overall survival rate for the 390 study participants, who were randomized 2:1 to either velimogene aliplasmid or physician's choice of chemotherapy (dacarbazine or temozolomide; NCT00395070). No new trials are planned for velimogene aliplasmid.

Bacille-Calmette-Guerin

Bacille-Calmette-Guerin (BCG) has been historically used in intralesional therapy, but it has a severe adverse event profile. The aim of using BCG for intralesional therapy against metastatic melanoma is to stimulate an immune reaction to eliminate the tumor using the patient's own immune system.[28] BCG is a live, attenuated strain of Mycobacterium bovis, which is an antigen that can trigger an immune reaction. In animal models, BCG produces a nonspecific immune response.[28] In humans, it has been used for intralesional therapy in patients who have already demonstrated an immune reaction to BCG to stimulate an immune response against the injected lesion.[28] Adverse events include fevers, chills, diaphoresis, arthralgias, malaise, and angioedema in patients positive for tuberculin and those with lymphadenopathy, pneumonitis, BCG granulomas, and granulomatous hepatitis.[21,28-30] Toxicity is caused by the patient having an immune response to BCG; thus, patients who have no immunity against BCG cannot demonstrate adverse events.

Seigler et al[29] recruited 160 patients with locally recurrent melanoma who were treated with intralesional BCG using a 4-stage approach. In the first stage, participants who were immune sensitive to BCG were selected; in the second stage, a delayed hypersensitivity reaction to BCG was stimulated in participants with booster therapy; in the third stage, adoptive immunity was achieved by harvesting participant lymphocytes, which were exposed to tumor cell samples and reinjected into the participants; and, in the fourth stage, to further increase antitumor responsiveness, the participants were injected with a vaccine of tumor cells and BCG.[29] Of the 70 study patients evaluated in stage I, 44% (31) were sensitive to BCG, and, as those study patients progressed through the four stages, they demonstrated increased rates of antitumor immune responsiveness.[29] Of the 62 participants examined for cell-mediated, tumor-specific immunity, 69% (n = 43) had a prolonged response, with 60% mean tumor lysis.[29]

Table 1 - Select studies of intralesional therapies[a]

Author	Treatment	Documented bystander effect	No. of Participants	Dosing	Dosing interval	Treatment duration	CR %	PR %	SD %	PD %
Bedikian[16]	Velimogene aliplasmid	Yes	127	0.5-2 mg	Once weekly	6 wk	3	9	25	63
Stopeck[19]	Velimogene aliplasmid	No	51	10 μg	Wk 1-4, 8, 9	≤ 6 cycles	2	16	24	59
Gonzalez[27]	Velimogene aliplasmid	No	77	10 μg	Once weekly/6 wk	≤ 3 cycles	3	7	23	68
Karakousis[28]	BCG	No	8	0.1 mL of 4×10^{11} to 9×10^{11} viable organisms/mL	n/a	Once	75	0	0	25
Kidner[41]	BCG/imiquimod	No	19	3 × 106 cfu/5%	5-7 d/wk every 2 wk	2 injections titrated to local inflammation	56	11	33	0
Marty[33]	ECT/Bleo	No	41[b]	≤ 1000 IU/cm3, depending on tumor size	n/a	Once	73	11	11	5
	ECT/Cis			≤ 2 mg/cm3, depending on tumor size						
Byrne[20]	ECT/Bleo vs. Bleo vs. ECT	No	19	1 U/mL tumor volume	4, 8, or 12 wk	4, 8, or 12 wk	72	5	18	5
Heller[32]	ECT/Bleo vs. Bleo vs. electroporation	No	34	0.025 U, 1250 V/cm	Once	Once	89	10	1	0

Table 1 - Select studies of intralesional therapies[a] (continued)

Reference	Treatment	Documented bystander effect	No. of Participants	Dosing	Dosing interval	Treatment duration	CR %	PR %	SD %	PD %
Mir[31]	ECT/Bleo	No	20	18 or 27 U/m^2, 1300 V/cm	Once	Once	53	39	8	0
Ridolfi[34]	GM-CSF, IL-2	No	16	150 ng, 3 MIU	Every 21 d	6 cycles	0	13	69	19
Boyd[46]	IL-2	No	39	10.4 MIU	Biweekly	4 cycles	51	31	18 (SD/PD)[c]	
Weide[18]	IL-2	No	48	0.3-6.0 MIU	3 × wk	1-32 wk	69	NR	NR	NR
Thompson[23]	10% rose bengal	Yes	80	NA	Wk 1, 8, 12, 16	≤ 4 cycles	26	25	18	31
		Yes	20	NA	Once	1 cycle	20	20	35	25
Senzer[38]	TVEC	Yes	50	106 PFU first dose, then 108 PFU	First interval 3 wk, then every 2 wk	≤ 24	16	10	24	50
Andtbacka[39]	TVEC	No	295	106 PFU first dose, then 108 PFU	First interval 3 wk, then every 2 wk	NR	11	16	73 (SD/PD)[c]	
	GM-CSF	No	141	125 μg/m^2	Daily × 14 d every 4 wk	NR	1	5	94 (SD/PD)[c]	

Bleo: bleomycin; Cis: cisplatin; CR: complete response; ECT: electrochemotherapy; GM-CSF: granulocyte macrophage colony-stimulating factor; IL-2: interleukin-2; n/a: not applicable; NR: not reported; PD: progression of disease; PFU: plaque-forming unit; PR: partial response; SD: stable disease; IU: international units; MIU: million international units; TVEC: Talimogene laherparepvec.

[a]Only studies with sufficient data regarding responses are included.

[b]Multiple tumor types are included, but responses are not split for study patients with melanoma and without melanoma. Bleo/Cis is equally effective.

[c]Responses were not split out.

Table 2 - Common adverse events from intralesional therapies

Type of Therapy	Adverse Events
Bacille-Calmette-Guerin[21,28-30]	• Angioedema (with positive tuberculin test) • Arthralgia • Bacille-Calmette-Guerin granulomas • Chills • Diaphoresis • Disseminated intravascular coagulation • Granulomatous hepatitis • Lymphadenopathy • Malaise • Pneumonitis
Electrochemotherapy plus bleomycin or cisplatin[22,35]	• Pain at injection site • Ulcerations
GM-CSF[34]	• Flulike symptoms
Interleukin-2[18,36]	• Flulike symptoms • Injection site pain/erythema
Rose bengal 10%[12,13,23,37]	• Blistering • Edema • Headache • Local pain • Inflammation • Pruritus • Skin discoloration • Vesicles
Talimogene laherparepvec[9,39,40]	• Cellulitis • Chills/rigors • Fatigue • Pyrexia
Velimogene aliplasmid[16,19,24-27]	• Asthenia • Fatigue • Flulike symptoms • Injection-site pain • Myalgia • Paresthesia • Rigor

GM-CSF: granulocyte macrophage colony-stimulating factor

Of the 19 study patients who never developed immunity against melanoma, all of them progressed and died of complications from diffuse, distant metastatic disease.[29] Although results from early clinical trials correlated well with the rationale for BCG intralesional therapy, the adverse event profile of BCG is a limitation to its broad implementation.[21,28-30] And, although BCG uses M. bovis to stimulate an immune, antitumor response, it also produces complications associated with that same immune response, leading to adverse events and disseminated intravascular coagulation at a rate of 12%.[45] Because of these inflammatory reactions and the concomitant high risk of morbidity, BCG treatment requires that patients be closely observed. Prophylactic treatment should be provided, such as antihistamines and isoniazid, because of the morbidity of these adverse events.[30] In addition, to minimize the morbidity of these reactions when they do occur, signs or symptoms of these complications should be treated with hydration, antituberculosis therapy, steroids, antihistamines, and supportive care.[30]

Electrochemotherapy

Electrochemotherapy (ECT) is used as an intralesional therapy that delivers agents into the treated lesion. ECT applies high-intensity, pulsed electrical current to the treated lesion that renders the tumor cells permissive for the uptake of drugs, viruses, or genetic material.[31,46] By contrast, electroporation delivers the current to the lesion without the need of additional agents. Therefore, ECT can be used to deliver therapeutic agents.

Of all the agents used in combination with ECT, bleomycin is the most commonly reported (0.025 units delivered with ECT at 1250 V/cm).[32] ORRs up to 98% have been reported and CR in more than 50%; however, case series have been small and limited by short follow-up periods.[33] No significant adverse events have been noted.[22,35] Marty et al[33] conducted the European Standard Operating Procedures of Electrochemotherapy study, based on the experience of leading European cancer centers, that has been a landmark trial in the field.[33] Prior to the report by Marty et al.,[33] which was published in 2006, different study groups used a variety of protocols with different pulse parameters, pulse generators, electrode types, and dosages of chemotherapy. Marty et al[33] generated standard operating procedures in a prospective study with two years of follow-up using bleomycin or cisplatin. For bleomycin, they used either intravenous 15,000 IU/m^2 in a bolus lasting 30 to 45 seconds or various intratumoral doses, depending on the tumor size. Cisplatin was administered based on tumor size.[33] Depending on the number of nodules treated, study participants either received local anesthesia or general anesthesia.[33] Procedures were performed on an outpatient basis or during a one day admission.[33] Using 5000 Hz electric pulses was more effective than using 1 Hz.[33] Melanoma nodules showed a lesional response of 80% and a CR rate of 66.3%.[33]

Subsequently, a meta-analysis of 44 studies analyzed intralesional treatment with ECT on 1,894 lesions.[46] Results were reported for both bleomycin and cisplatin.[46] When the clinical responses in all histological diagnoses were evaluated, the CR rate was 59.4% and the ORR was 84.1%.[46] When the melanoma results were evaluated, the rate of CR and ORR of treated melanoma tumors were 56.8% and 80.6%, respectively.[46] No adverse events were reported.[29]

Although these results are encouraging, the data are limited due to their small size and lack of long-term follow-up. Therefore, further studies are required to determine which patients may benefit from ECT.

Granulocyte macrophage Colony-Stimulating Factor

Use of Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) for intralesional therapy against metastatic melanoma is based on two mechanisms.[47] GM-CSF stimulates dendritic cells that then induce antitumor immune responsiveness.[47] The result is twofold: direct destruction of the injected lesion and enhanced antigen presentation, leading to an immune response against metastatic melanoma. T cells treated with GM-CSF have demonstrated increased antitumor responsiveness.[47] Reported adverse events have generally been tolerable and typically constitute flulike symptoms.[9,34,47]

In addition to increasing the antitumor responsiveness of T cells, GM-CSF also appears to reduce the immune-inhibitory effects of metastatic melanoma by having an effect on the cells implicated as mediators of decreasing the immune response against cancer.[9,47] GM-CSF has been shown to decrease T-regulator, suppressor, and myeloid-derived suppressor cells, which are all mediators of decreased T cell antitumor activity.[9,47] Patients with a higher T cell composition of the tumor infiltrate with higher interleukin-2 (IL-2) receptor expression are more likely to demonstrate a clinical response to therapy.[9,47] Phase 1 data showed increased CD4, CD8, lymphocyte, histiocyte, and eosinophil tumor infiltrate in the injected lesions and a higher likelihood of clinical response in patients with a higher T cell composition of the tumor infiltrate with a higher IL-2 receptor expression.[48] Phase 1/2 studies showed ORRs up to 26%.[34,35,48] Efforts are underway to further evaluate mechanisms to enhance the immune response against melanoma.

Interleukin-2

IL-2 is a naturally occurring glycoprotein secreted by T cells to augment the immune response and was first used in clinical cancer studies in the early 1980s.[49] This glycoprotein promotes T lymphocyte proliferation and stimulates cytotoxic T cells and natural killer cells.[50] IL-2 has been used as immunotherapy for nearly 40 years, although it has mostly been employed as an intravenous agent.[50] Its use for intralesional therapy is limited due to logistical problems because patients require multiple injections per lesion and IL-2 is costly.[50]

The immune-stimulating mechanism of IL-2 has already been applied to melanoma and other solid tumors as a systemic therapy.[50-52] It produces a relatively high rate of morbidity when considering its relatively low response rates, which range from 10% to 15%.[52] Because IL-2 has the potential to induce durable responses, high-dose systemic IL-2 was the mainstay for the treatment of tumors like melanoma and renal cell carcinoma up until the 2000s.[51,52] Although its usage has recently tapered off as more effective drugs are now available, IL-2 is still considered a treatment option for unresectable melanoma.[51,52] Treatment of tumors has been reported using intralesional and perilesional injections of IL-2, whereby an IL-2 injection into the tumor has been shown to be effective.[53] Intralesional IL-2 has been studied in many forms, including use as viral vectors, xenogeneic monkey fibroblasts, and IL-2 cultured lymphocytes harvested from patients with melanoma, as well as adjunctive therapy with other systemic therapy and topical agents.[17,49,54-57] Response rates were low and erratic until human recombinant IL-2 was developed, which has provided consistent and promising results.

Unlike systemic IL-2, which has a morbid adverse event profile, intralesional IL-2 typically produces flulike symptoms alone.[36] Local adverse events such as injection-site pain and erythema have also been reported.[12,13,18,23,36,37] The number of study patients in published reports has been small: 7 participants treated in one documented case series and 23, 39, and 48 study patients in three phase 2 studies.[18,39,58,59] Response rates consistently exceed 80%.[36,58,59]

Boyd et al[36] reported improved overall 5-year survival rates in study patients with CR (51% of 39 patients) and study patients with PR (21% of 39 patients). The reported 5-year survival rates were 80% and 33%, respectively.[36] Complete responders had a significant overall survival benefit when compared with partial responders ($p = 0.012$).[36] Despite demonstrating a high response rate with minimal rates of morbidity, IL-2 has not demonstrated a significant bystander effect, despite its immune-mediated mechanism.[36] Studies so far conducted have used an onerous administration scheme requiring multiple injections each week; furthermore, because IL-2 is a costly drug to purchase, it is not broadly pursued in research.[36]

Rose Bengal

Rose Bengal (PV-10) is an investigational agent for use as an intralesional therapy. The 10% rose bengal solution is a water-soluble stain used to diagnose liver and eye cancers and ocular damage, as well as in food coloring in Japan and as an insecticide, with medical reports being published as early as the 1920s.[37,60] Because of the wide variety of its application, experience with the drug is extensive, and its safety profile has been well established.[12,13,23,37] As a xanthine dye, the hypothesized mechanism of action of 10% rose bengal is that it creates reactive oxygen by reacting with visible and ultraviolet light, thereby mediating phototoxic reactions. It is selectively absorbed by lysosomes of cancer cells, inducing autolysis,[61,62] and 10% rose bengal is currently under investigation for melanoma and liver tumors (NCT00986661, NCT02557321, NCT02288897).[12,13,23,37] Responses have been reported in study patients refractory to previous systemic ipilimumab, anti-programmed death ligand 1 antibody and vemurafenib, and therapeutic responses have been seen in study patients progressing after a median of six treatments.[12,23]

A bystander effect has been observed in 10% rose bengal.[23,62] Use of 10% rose bengal leads to increased tumor-specific interferon-α secretion in a mouse model, induces an increase in circulating, cytotoxic $CD3^+/CD8^+$ T cells, and recruits dendritic cells to drain lymph nodes.[12,62] Injection into the non-tumor-bearing flanks of mice had no effect on distant lesions.[62] Rather, the agent must be injected into a tumor lesion to induce a bystander effect. The rate of morbidity is generally considered to be low, although most patients report some local adverse events, most commonly pain (≤80%).[60] Local blistering (40%) has been correlated with a

better outcome.[60] Other reported adverse events include vesicles, edema, skin discoloration, inflammation, headache, and pruritus around the treatment site.[60]

The first phase 1 trial of 10% rose bengal included 11 study patients.[37] Treatment with 0.5 mL/cc per lesion induced an ORR in more than one half of participants (both CR and PR: 27%).[37] The effect was dose-dependent, as target lesions receiving less than 1.2 mL 10% rose bengal had a significantly lower response rate than lesions receiving a higher dose (25% vs. 69%).[37] A bystander effect was seen in 27% of the study patients and correlated with the response of the injected lesion.[37] In another phase 1 trial, Thompson et al[23] enrolled 20 patients, injecting a single dose of 10% rose bengal in up to 20 lesions per participant. Response rates were comparable with those seen in the first phase 1 study.[23,37] ORR was achieved in 40% of study patients, including a 20% complete response rate, and a bystander effect was reported in 15% of study patients.[23]

Thompson et al[23] injected up to 20 lesions per study patient at day 0 and repeated the injection if needed after 8, 12, and 16 weeks. A total of 80 study patients were included, the majority of whom responded after fewer than two injections, resulting in an ORR of 51%, of which the CR rate was 26%.[23] A bystander effect was seen in 40% of 35 evaluable study patients and was correlated with the response of injected lesions (CR rate, 31%; PR rate, 9%).[23] Both visceral and cutaneous lesions were susceptible to this effect.[23] Overall responses were correlated with initial treatment of all discernible disease, with a CR rate seen in 50% of study patients for whom all baseline disease was treated; CR was not seen in study patients with stage 4 melanoma.[23]

Based on these results, expanded access of this trial became available (NCT02288897). As of publication, more than 100 patients with melanoma have been enrolled in this trial. In the phase 3 trial, patients with stage IIIB/IIIC/IVa disease will be randomized 2:1 to either 10% rose bengal or systemic chemotherapy, allowing crossover, with progression-free survival as the study's primary end point.

Talimogene Laherparepvec

Talimogene Laherparepvec was approved by the US Food and Drug Administration in 2015.[63] It shows a trend toward improved survival rates and a robust bystander effect.[39] Talimogene laherparepvec is an oncolytic, immune-enhanced herpes simplex virus type 1, and its various genetic modifications include deletions of ICP34.5, ICP47, and insertion of GM-CSF. Oncolytic viruses like talimogene laherparepvec are designed to selectively multiply in tumor cells.[64] At least nine virus groups are being investigated in clinical trials.[65] Oncolytic viruses have direct effects on the metabolic processes of cancer cells. They selectively replicate in tumors, thereby destroying and infecting cancer cells due to their direct effects on the metabolic processes in the cell as well as their ability to induce immune responses that target the cancer cell. This action is thought to be aided by the activation of nuclear factor κB and the release of chemokines and cytokines from the cancer cell.[65] Oncolytic viruses demonstrate limited systemic applicability due to the immune responses of the host, but they are suitable for intralesional injection. Specifically with talimogene laherparepvec, ICP47 deletion helps to prevent blocking antigen presentation and enhances virus growth and replication in tumor cells.[38,66] Replacing the coding sequence for neurovirulence factor ICP34.5 with the human cytokine GM-CSF enables talimogene laherparepvec to initiate a systemic antitumor response by enhancing immune response to tumor antigens.[66] The most common adverse events seen with this agent are fatigue, chills, and pyrexia.[49]

Senzer et al[38] investigated the effectiveness of talimogene laherparepvec in study patients with stages III (n = 10) and IV (n = 40) melanoma in a single-arm, phase 2 trial. Study patients received intralesional injections of either talimogene laherparepvec or GM-CSF.[38] The initial injection had a volume of up to 4 mL of 10^6 pfu/mL followed three weeks later by 4 mL of 10^8 pfu/mL, every two weeks, for up to 24 treatments.[38] The protocol allowed injection with or without ultrasonographic guidance and included cutaneous, subcutaneous, and nodal lesions. An ORR based on Response Evaluation Criteria In Solid Tumors was 26% (CR rate, 8%; PR rate, 5%).[38] After one and two years, the overall survival rates were 58% and 52%, respectively.[38]

Based on these data, a phase 3 study was conducted.[14,39] This study randomized 436 patients 2:1 to intralesional talimogene laherparepvec (n = 295) or subcutaneous GM-CSF (n = 141) and used the same talimogene laherparepvec regimen as the phase 2 trial.[39] The ORRs were 26.4% for those assigned to talimogene laherparepvec and 5.7% for those assigned to GM-CSF.[39] The results showed a significant difference in durable response rates (i.e., PR or CR rate for >6 months), with 16.3% in the talimogene laherparepvec group and 2.1% in the GM-CSF group ($p < 0.001$); durable response rates were higher in study patients with stage IIIB/C melanoma (33% for the talimogene laherparepvec group vs. 0% for those in the GM-CSF group).[39] Six previously unresectable study patients were converted to resectable. Fewer than 3% of study patients experienced grade 3/4 adverse events.[39] For the entire patient population, the overall survival rates trended toward statistical significance (23.3 months for the talimogene laherparepvec group vs. 18.9 months for the GM-CSF group; $p = 0.0051$).[39]

A subgroup analysis showed survival benefit in patients with stage IIIB/C and IV M1a disease, and the effect was stronger when talimogene laherparepvec was given as first-line therapy as opposed to second-line therapy or higher.

A lesion-level analysis of the phase 3 trial of 3,219 lesions in 286 patients showed a 50% reduction in 64% of the injected lesions, 32% of the uninjected nonvisceral lesions, and 16% of the uninjected visceral lesions.[14] These findings indicate a bystander effect and, thus, a systemic immune response from the local injection of talimogene laherparepvec.[14]

A phase 1b study of talimogene laherparepvec added to ipilimumab in 19 participants suggested a higher CR rate for the combination than for either agent alone.[40] Grade 3/4 adverse events occurred in 32%.[40] Two study patients had possible immune-related grade 3/4 adverse events, and, of the 17 study patients with investigator-assessed response, the ORR was 41% (CR rate, 24%; PR rate, 18%) and stable disease was seen in 35%.[40] Median time to response was 2.9 months (NCT01740297).

Topical therapies

Topical therapies have shown some success in superficial lesions and are generally associated with low rates of morbidity.[41,67-70] Typically, they are better suited for thinner lesions. Topical diphencyprone cream is a synthetic contact sensitizer that has been used to treat alopecia and warts.[71,72] The largest trial to date was conducted by Damian et al.,[67] who studied 58 patients, 50 of whom were treated for more than one month. A total of 46% achieved CR and 46% achieved PR; however, the results of this study should be interpreted with caution, as the majority of results came from the same research group.[67-70]

Imiquimod is a toll-like receptor agonist approved by the US Food and Drug Administration for the treatment of genital warts, keratosis, and superficial basal cell carcinomas.[73] A treatment regimen for melanoma has not been established, as the application of imiquimod ranges from once weekly to twice daily and from 2 to 88 weeks.[74] Since 2000, it has been used for advanced melanoma in various case reports and small case series.[6,75-77] The largest case series is of five patients treated with combination topical imiquimod/fluorouracil; a response was elicited in 44 of 45 lesions.[77] Combined treatments with IL-2 and BCG have also been reported.[41,57] More evidence is available for patients with lentigo maligna, including a large case reporting that more than 90% of study patients with lentigo maligna experience regression with daily or twice-daily application of an imiquimod cream.[74,78]

Conclusions

The standard of care for patients with locoregional advanced or metastasized melanoma is to render a patient free of disease as long as the disease is sufficiently limited. When this is no longer feasible, intralesional therapy is a possible option due to its good local response and tolerable adverse event profile, as well as the option to provide outpatient treatment. A bystander effect observed in various agents adds to its appeal. During the last few decades, other agents have been tested for intralesional therapy with varying success. Many intralesional compounds now available produce a broad range of local response rates. The ideal agent should have a low toxicity profile, be easy to administer, lead to fast responses, and trigger a systemic immune response, thereby creating a bystander effect. These criteria were predominantly met in the results of trials using 10% rose bengal and talimogene laherparepvec in up to 40% of study patients.

Most agents (Bacille-Calmette-Guerin, interferon, granulocyte macrophage colony-stimulating factor) demonstrated inconsistent rates of efficacy, but the treatment field changed when velimogene aliplasmid, 10% rose bengal, and talimogene laherparepvec were introduced. Velimogene aliplasmid did not meet its primary endpoint in a phase 3 trial, but talimogene laherparepvec did meet its phase 3 trial objectives, demonstrating a survival benefit in select study patients. The results of phase 2 results of 10% rose bengal trials are also promising and a phase 3 is still recruiting (NCT02288897). Other options include combinations of intralesional therapies and systemic therapies, including ipilimumab/talimogene laherparepvec and pembrolizumab/rose bengal (NCT02557321).

Our treatment approach should be individualized per patient, based on the extent of disease, tumor characteristics, and disease-free interval, as well as patient characteristics such as age, performance status, and co-morbidities, and work to maintain quality of life for as long as possible. An appropriate approach is often not a single therapy but rather a combination of injectable treatments, regional perfusion therapies, and systemic therapies.

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part II

BRAF treatment in advanced melanoma

6

BRAF inhibition for advanced locoregional BRAF V600E mutant melanoma: a potential neoadjuvant strategy

Abstract

Selective BRAF inhibitors (BRAFi) yield objective responses in 50% of patients with metastatic BRAF V600E mutant melanoma. Adding a MEK inhibitor increases this response rate to 70%. Limited data are available on the outcomes of unresectable stage III patients, and it remains unclear whether BRAF-targeted therapy can be utilized as a neoadjuvant strategy. Data on patients with advanced locoregional BRAF V600E mutant melanoma treated with BRAF-targeted therapy at Moffitt Cancer Center were analyzed to determine response rates, subsequent resection rates after tumor downsizing, pathologic responses, and patient survival. Fifteen patients with locoregional disease treated with BRAF-targeted therapy, either BRAFi alone (vemurafenib; 11 patients) or a combination of a BRAFi and a MEK inhibitor (dabrafenib plus trametinib or placebo; four patients), were identified. The median age was 50 years; the median follow-up was 25.4 months. The median BRAF-targeted therapy treatment duration was 6.0 months (range 1.2-29.4 months). Response Evaluation Criteria In Solid Tumors-based evaluation demonstrated objective response in 11 patients (73.3%). Six patients underwent resection of the remaining disease after therapy. Pathological analysis showed complete pathologic response (n = 2), partial pathologic response (n = 2), or no pathologic response (n = 2). Four of six patients undergoing surgery have been alive for more than two years, including three patients currently free from active disease. No complications attributable to BRAF-targeted therapy were observed in the perioperative period. Dose reduction or discontinuation because of toxicities occurred in 10/15 patients. Neoadjuvant BRAF-targeted therapy may be effective in advanced locoregional BRAF V600E mutant melanoma patients in increasing resectability, yielding pathological responses, and achieving prolonged survival.

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Introduction

Of the 73,870 cases of melanoma estimated to be diagnosed in the USA in 2015, ~9% of patients present initially with regional lymph node or in-transit metastases.[1] Data from the Multicenter Selective Lymphadenectomy Trial I of patients with resected 1.2-3.5 mm deep melanomas demonstrated a 10% locoregional recurrence rate in those who underwent sentinel lymph node biopsy and 19% in those who underwent observation of the nodal basin.[2,3] Most locoregional disease is amenable to resection aiming to render the patient free from disease.[4] However, some patients present with unresectable bulky adenopathies because of surgical limitations such as involvement of neurovascular structures. Similarly, as many as 24% of patients with recurrent locoregional melanoma have satellite and/or in-transit disease not amenable to complete resection.[5] Radiation, intralesional injection, hyperthermic isolated limb perfusion, and isolated limb infusion may be of benefit to some patients, but the majority of patients with locoregional melanoma recurrence ultimately require systemic therapy.[6,7]

Several phase 2 clinical trials have been conducted in resectable stage III melanoma patients using neoadjuvant systemic therapy, including temozolomide, high-dose interferon, or biochemotherapy.[8-10] Objective response rates are suboptimal for use as a neoadjuvant strategy in patients with unresectable stage III disease. BRAF-targeted therapy may represent a more effective means for tumor debulking/cytoreduction and subsequent definitive surgery. Phase 3 studies of BRAF inhibitors vemurafenib and dabrafenib have shown objective response rates of 50% in metastatic BRAF V600E mutant melanoma patients, with ~90% of patients showing tumor regression on waterfall plots.[11,12] Even higher objective response rates (up to 68%) are achievable with strategies combining a BRAF inhibitor (BRAFi) and an MEK inhibitor (MEKi).[13-17] Although unresectable stage III patients comprised 2-9% of the cohorts in these studies, no data have been reported on response rates, conversion rates to the resectable state, and tolerability for this subpopulation.

To address outcomes of unresectable stage III melanoma treated with BRAF-targeted therapy, we retrospectively analyzed data on advanced locoregional BRAF V600E mutant melanoma patients treated with BRAFi or BRAFi/MEKi.

Materials and methods

After approval by the Institutional Review Board of the University of South Florida, data on patients treated with BRAF-targeted therapy (vemurafenib or dabrafenib ± trametinib) for unresectable locoregional BRAF V600E mutant melanoma at Moffitt Cancer Center from 2011 to 2013 were collected. Patients were systematically identified through BRAF test results, pharmacy prescription records, protocol enrollment, and survey of surgical and medical oncologists in the Department of Cutaneous Oncology. Patients with unresectable locoregional disease, defined as in-transit metastases, bulky adenopathies that could not be resected without compromise of neurovascular structures, or regional lymph node metastases that were beyond standard surgical parameters (e.g. axillary disease with chest wall invasion), were included. Patients were excluded if they did not receive initial full dose levels of vemurafenib (960 mg orally twice daily) or dabrafenib (150 mg orally). Demographic and baseline data collected included sex, age, location and extent of disease, serum lactate dehydrogenase (LDH) level, and type of BRAF-targeted therapy received. Data on clinical outcomes included duration of systemic treatment, best radiographic response as measured by Response Evaluation Criteria In Solid Tumors (RECIST 1.1 on computed tomography, PET/computed tomography, and/or MRI), toxicities, surgical outcomes, and survival.

When surgery was performed, the resected specimens were analyzed for pathologic response. Pathologic parameters assessed included percentage of viable tumor and presence of necrosis. Pathologic response was graded as follows: (i) complete pathologic response if no viable tumor cells were observed, (ii) partial pathologic response if 10-99% of the tumor area was necrotic but still contained viable tumor cells, and (iii) no pathologic response if less than 10% of the tumor was necrotic/ regressed. As the data are exploratory in nature, the results are presented in a descriptive manner. GraphPad Prism 6.02 (GraphPad, La Jolla, California, USA) and IBM SPSS 21.0 (SPSS Inc., Chicago, Illinois, USA) were utilized for analyses.

Results

Baseline characteristics

A total of 15 patients met the inclusion criteria. The median age at initiation of BRAF-targeted therapy was 50 years; nine patients were male, and most patients had advanced nodal disease alone (12 patients) or in combination with in-transit disease (two patients). No patient had received prior systemic therapy for unresectable disease. BRAF genotyping was performed by pyrosequencing (12/15) and real-time PCR/Cobas (2/15). For the remaining patient, genotyping was performed at an external institution and the method was unspecified. Eleven patients received vemurafenib alone, three patients received dabrafenib plus trametinib (clinicaltrials.gov: NCT01072175), and one patient received dabrafenib with either trametinib or placebo on a blinded clinical trial (clinicaltrials.gov: NCT01584648). LDH levels at the start of treatment were available for 10/15 patients, with 4/10 patients having an LDH level above the normal limit. BRAF-targeted therapy was not restarted after surgery unless the disease progressed.

Patient outcomes

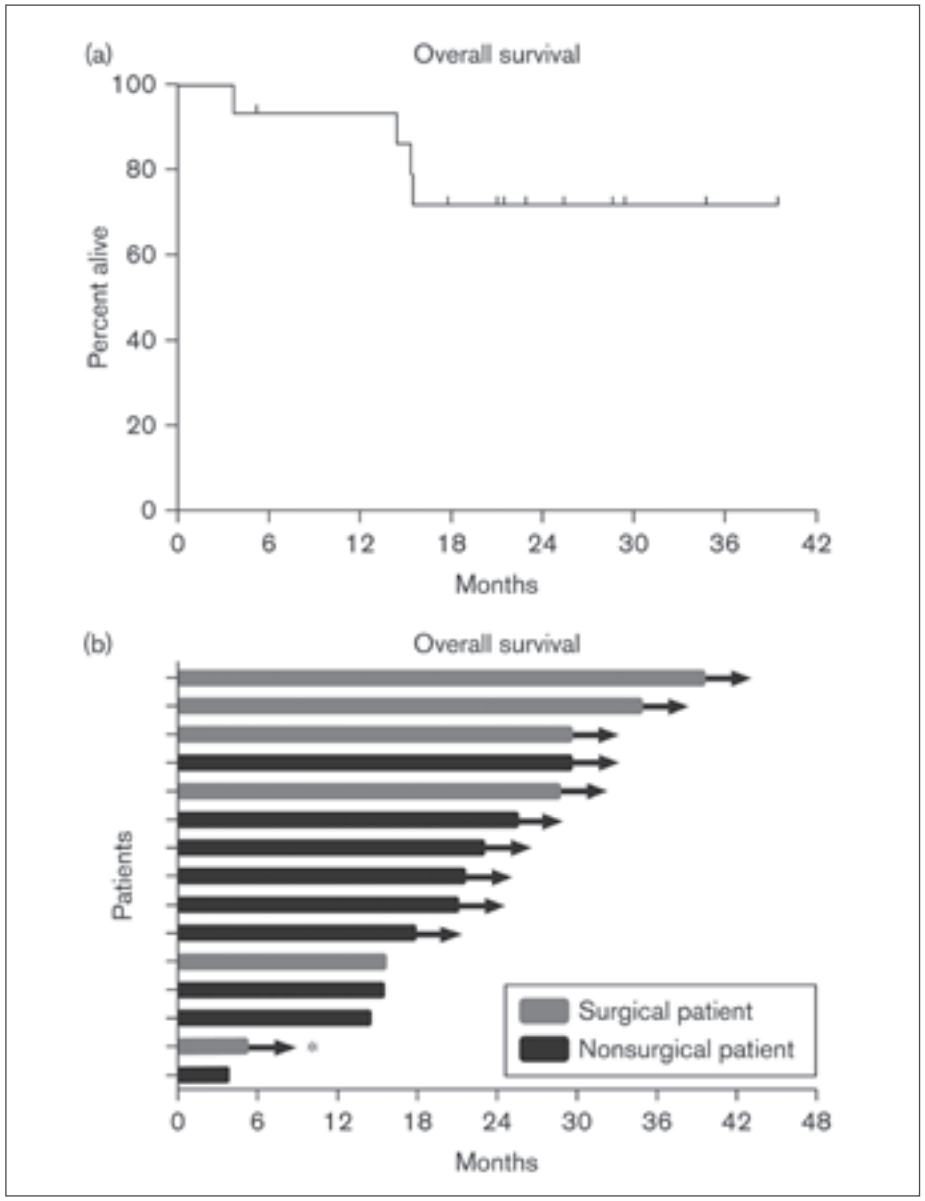
The median follow-up was 25.4 months from initiation of BRAF-targeted therapy. RECIST-based evaluation of the best overall response demonstrated an objective response in 11 patients (73.3%; one complete response, ten partial responses). Two patients had stable disease and two patients had progressive disease. Patients received BRAF-targeted therapy for a median duration of 6.0 months (range 1.2-29.4 months). Two patients remain on active treatment with BRAF-targeted therapy. Reasons for discontinuation include recommendation for surgical resection of disease (4/13), toxicity (2/13) and disease progression (7/13). Of the 11 patients treated with vemurafenib, eight patients required a dose reduction because of toxicities (fatigue, rash, hand-foot syndrome, arthralgias, elevated transaminases). Two of four patients treated with dabrafenib/trametinib discontinued therapy after less than two months because of acute uveitis and a combination of arthralgias, fevers, and rash, respectively. Six patients with partial response underwent resection of residual disease after a median time on BRAF-targeted therapy of 4.7 months (range 1.2-8.9 months). In the nine patients not undergoing resection of the disease, the median treatment duration was 6.0 months (range 2.3-29.4 months). A total of five patients died of the disease, and the median overall survival by Kaplan-Meier evaluation has not yet been reached (Figure 1). The estimated 2-year survival was 68%.

All six patients undergoing surgery after BRAF-targeted therapy were rendered grossly free from disease. All LDH levels were within the normal range after BRAF-targeted therapy before surgery. The median time from discontinuation of BRAF-targeted therapy to surgery was 20 days (range 5-227 days). No unexpected complication occurred during surgery nor in the postoperative period that could have been attributed to prior exposure to BRAF-targeted therapy. Of the six patients documented to be alive past 24 months, four discontinued therapy and underwent surgical resection. Two patients have not relapsed 24.9 and 39.5 months post resection. Interestingly, both patients received dabrafenib plus trametinib for 1.2 and 1.4 months, respectively, before discontinuation because of toxicity. One additional patient has remained free from active disease for 25.6 months from his surgery after receiving stereotactic radiosurgery for two brain metastases two months after his nodal resection. The fourth patient had disease relapse and underwent craniotomy for a brain metastasis, followed by several lines of systemic therapy (ipilimumab, chemotherapy, nivolumab). Of the remaining two surgical patients, one succumbed to widespread disease recurrence despite restarting vemurafenib and the other was lost to follow-up.

Overall survival

Histopathologic evaluation of resected disease after BRAF-targeted therapy demonstrated partial to complete pathologic response in four of six patients (Figure 2). In two patients, complete pathologic response was observed; one had no melanoma cells within the 51

Figure 1 - Overall survival of unresectable locoregional BRAF V600E mutant melanoma patients treated with BRAF-targeted therapy



(a) Kaplan-Meier curve of overall survival for all patients from the time of BRAF-targeted therapy initiation. At a median follow-up of 25.4 months, the median overall survival was not yet reached (b) Swim plot of overall survival for all patients from the time of BRAF-targeted therapy initiation. Surgical patients discontinued therapy and underwent resection of residual disease, whereas nonsurgical patients remained on therapy. The arrow (→) indicates ongoing survival.
** Lost to follow-up*

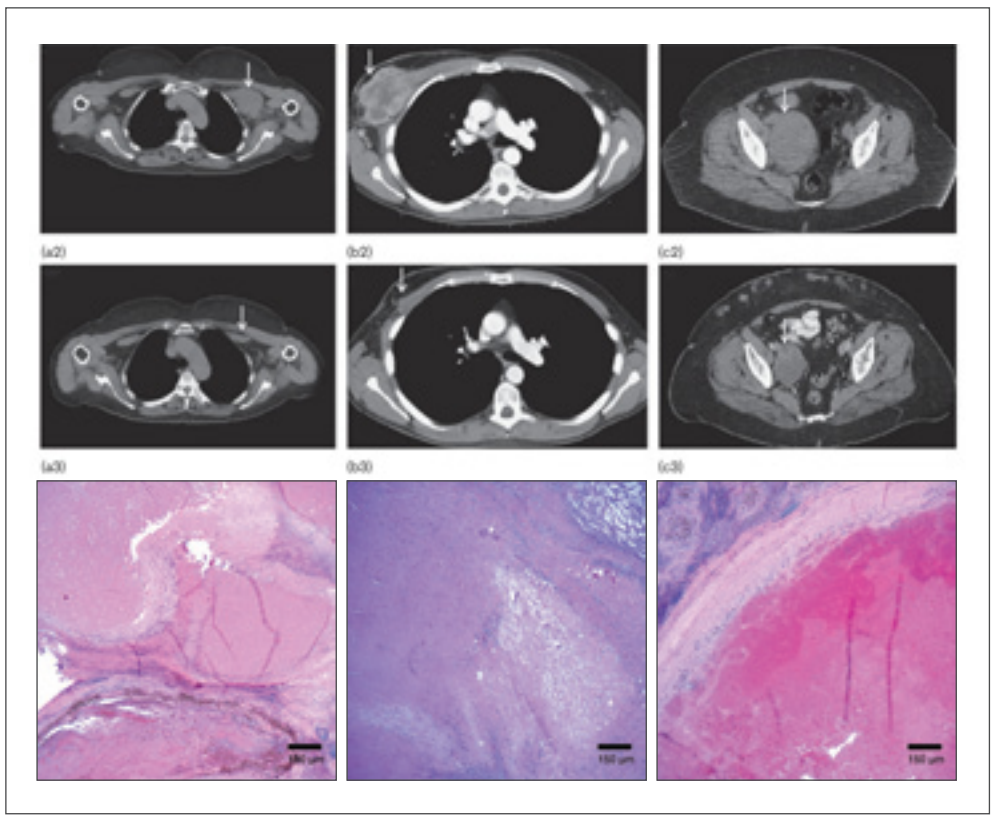
axillary nodes examined and the other had no viable melanoma cells in nine pelvic lymph nodes and subcutaneous fat, although some nodes exhibited nodular aggregates of melanin-laden macrophages and necrosis. Two patients had a partial pathologic response (defined as 10-99% necrosis). Of these, one patient had 15 axillary nodes resected, and two of these lymph nodes showed focal areas of viable tumor amidst abundant necrosis. The other patient had 28 axillary nodes resected, two of which had rare viable tumor cells in an otherwise necrotic background. Before BRAF-targeted treatment, this patient had three lymph nodes excised, demonstrating greater than 80% involvement by viable melanoma with extracapsular extension. The remaining two patients showed no pathologic response despite radiographic evidence of tumor shrinkage. One demonstrated tumor involvement of 75% of the total area and extracapsular extension in three of four pelvic lymph nodes; the other showed metastatic disease in 25 of 26 axillary nodes and 4/12 cervical nodes, with variable necrosis ranging from none to areas of complete necrosis, but averaging less than 10% of tumor necrosis. In the four patients with a partial or complete pathologic response, the resected tissue was characterized by large, geographic areas of necrosis and melanin deposition, both in the extracellular compartment and in the macrophages, rimmed by a lymphohistiocytic infiltrate (Figure 2). Within the necrotic regions, occasional ghost-like remnants of nonviable tumor cells could be seen.

Discussion

The role of neoadjuvant systemic therapy in advanced locoregional melanoma patients is not well defined. Past prospective studies with temozolomide, interferon, and biochemotherapy in resectable stage III patients have demonstrated tumor burden reduction and occasional pathologic complete responses.[8-10] However, response rates have been suboptimal and patients often experience significant toxicities. These studies did not include patients with unresectable melanoma. Several case reports of patients with metastatic BRAF V600E mutant melanoma given neoadjuvant vemurafenib have shown success in cytoreduction and subsequent resection of their disease.[18-20] Therefore, the use of a BRAF-targeted therapy as a neoadjuvant approach is an attractive, but largely untested, strategy to render patients surgical candidates and to achieve a disease-free status.

Our case series of 15 patients with unresectable, advanced locoregional BRAF V600E mutant melanoma treated with BRAF-targeted therapy showed an objective RECIST-based response rate greater than 70%. This response rate is on a par with that reported from the phase 3 studies of vemurafenib and dabrafenib ± trametinib, in which objective radiographic responses were seen in 50-70% of all metastatic BRAF V600E mutant melanoma patients.[11,12,21] Compared with the 16-26% objective radiographic response rates observed in the neoadjuvant studies of temozolomide and biochemotherapy in resectable stage III melanoma, the higher response rate with BRAF-targeted therapy likely represents a more effective strategy in both resectable and unresectable stage III melanoma patients.[8-10] In addition, six of 15 unresectable

Figure 2 - Pre-BRAF-targeted and post-BRAF-targeted treatment computed tomography scans correlated with hematoxylin and eosin stains of resected lymph node specimens



Three patients with radiologic partial responses after BRAF-targeted therapy. Patient A: target lesion in right axilla (a1), treated for 4.0 months (a2), resulting in a complete pathologic response. Post-treatment lymph node excision shows an area of necrosis, with cholesterol clefts peripherally and fibrosis centrally (a3). Patient B: target lesion in the left axilla (b1), treated for 8.8 months (b2). Post-treatment lymph node excision shows multiple geographic areas of necrosis, rimmed by fibrosis and a lymphocytic infiltrate, demonstrating no viable melanoma cells. Other areas of this lymph node showed rare viable tumor cells, indicating a partial response (b3). Patient C: target lesion in the pelvis (c1), treated for 1.2 months (c2), resulting in a complete pathologic response. Lymph node with complete tumor necrosis, showing a viable remaining lymph node (top left) and necrotic material rimmed by melanin-laden macrophages but no viable tumor cells (c3)

patients were able to undergo resection of their disease with curative intent after substantial cytoreduction, which further supports the use of neoadjuvant BRAF-targeted therapy.

Interestingly, pathologic specimens from patients with radiographic evidence of a partial response demonstrated both partial and complete pathologic responses. This suggests that patients who experience partial radiographic responses using standard RECIST criteria on BRAF-targeted therapy may actually have minimal to no viable malignant cells in measurable lymph nodes. Although not examined in this report, alternative methods of assessing BRAF-targeted therapy response, such as serial PET scanning and monitoring circulating free DNA BRAF V600E levels may more accurately correlate to pathologic findings.[22,23]

It remains to be seen whether improved survival will be achieved in unresectable locoregional BRAF-mutant melanoma patients undergoing neoadjuvant BRAF-targeted therapy followed by surgery as compared with BRAF-targeted therapy alone. There are limited data on the overall survival of unresectable BRAF-mutant stage III patients, but one would expect this to be less than the median of 2.5 years seen for all N3 patients (American Joint Committee on Cancer; ≥4 lymph nodes or matted lymph nodes involved by metastatic melanoma, or in-transit + lymph node disease metastases) among whom many are surgical candidates at presentation.[24] In our cohort of advanced locoregional BRAF V600E mutant melanoma patients, the median overall survival was not reached after more than two years of follow-up. Whereas four of six patients alive past two years underwent surgery, direct survival comparisons are not feasible because of the small sample size and retrospective nature of the study. However, the ability to remain disease-free off therapy is highly encouraging.

Conclusion

Our findings support the potential benefit of BRAF-targeted therapy in advanced locoregional BRAF V600E mutant melanoma patients, which can increase resectability and lead to pathologic partial and complete responses. Although toxicities and dose reductions/discontinuations were observed, these were similar to those in previous investigations and did not preclude surgical consideration. However, it should be acknowledged that definitive conclusions cannot be drawn from this study because of the small sample size and retrospective design. Multiple prospective clinical trials with neoadjuvant BRAFi plus MEKi strategies or actively enrolling BRAF-mutant melanoma patients with advanced locoregional disease have been planned (clinicaltrials.gov: NCT01972347, NCT02036086, NCT02303951, and NCT02231775). These studies will be valuable for confirmation of the clinical benefit of using a neoadjuvant BRAF-targeted approach. Furthermore, pathologic evaluation of tumors post treatment may provide prognostic information and an opportunity for molecular evaluation of patient-specific tumor responses.

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7

Long-term effects of BRAF inhibitors in melanoma treatment: friend or foe?

Abstract

The clinical development of selective BRAF inhibitors for metastatic BRAF V600 mutant melanoma patients has been a major breakthrough in targeted therapeutics. Objective response rates of approximately 50% have been observed in the phase 3 studies of the BRAF inhibitors vemurafenib and dabrafenib. The side effects can be relatively common, including proliferative skin toxicities. The latter range from hyperkeratosis and keratoacanthomas (KAs) to squamous cell carcinomas (SCCs) and new primary melanomas. In addition, case reports on the emergence of gastric/colonic polyps and RAS mutant malignancies have been described during BRAF inhibitor therapy. These events have been attributed to paradoxical activation of the MAPK pathway in BRAF wild-type cells exposed to selective BRAF inhibitors in addition to increased RAS activity. Combined BRAF and MEK inhibition appears to improve clinical outcomes and reduce cutaneous proliferation events as fewer KAs and SCCs have been observed with combination therapy. Next-generation pan-RAF inhibitors ('paradox breakers') and ERK inhibitors may further enhance clinical activity in metastatic BRAF-mutant melanoma patients and mitigate this paradoxical oncogenesis. Further investigation into the potential long-term effects of selective BRAF inhibitors is warranted as expanded use of these agents is expected in patients with BRAF-mutant melanoma and other malignancies.

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BRAF in melanoma

Although NRAS mutations were first described in melanoma in 1984, the development of targeted therapy for metastatic melanoma truly gained its footing with the identification of activating mutations in BRAF in 2002.[1] Mutations in exon 15 of the BRAF gene occur in 40-60% of cutaneous melanomas, with the most common being the V600E mutation.[2] This gain-of-function change leads to constitutive activation of the MAPK pathway (Figure 1a), resulting in increased cell growth, proliferation and invasiveness. Metastatic melanoma harboring BRAF mutations has been associated with worse overall survival prior to the development of targeted agents.[2] We have now seen the rapid development of selective BRAF and MEK inhibitors (BRAFi and MEKi, respectively) as targeted therapy for BRAF V600 mutant melanoma.

In the last three years, the US FDA has approved three targeted agents for metastatic BRAF-mutant melanoma patients (Figure 1b). Vemurafenib, a selective BRAF V600 mutant kinase inhibitor, was FDA approved in August 2011 based on the BRIM3 phase 3 study showing improved clinical outcomes compared to dacarbazine.[3] The objective response rate for vemurafenib was 48%, with a median progression-free survival (mPFS) of 5.3 months and an overall survival of 84% after six months. A second BRAFi dabrafenib was FDA approved in May 2013 after the randomized phase 3 trial (BREAK3) also confirmed superiority over dacarbazine. Dabrafenib yielded a response rate of 50%, with a mPFS of 5.1 months and an overall survival of 74% at six months.[4] Long-term follow-up for both studies has demonstrated mPFS over six months. More importantly, 26% of the patients are still alive three years after initiating treatment with BRAFi (vemurafenib), indicating that durable benefit is achieved in a subset of patients.[5] The third FDA-approved targeted agent is trametinib, a MEKi. However, a lower objective response rate (22%) and shorter mPFS were demonstrated with trametinib in the phase 3 METRIC trial as compared to data for vemurafenib and dabrafenib, making a BRAFi the preferred single agent BRAF V600 mutant melanoma targeted therapy.[6]

While vemurafenib and dabrafenib both have demonstrated clinical benefit, treatment-related adverse events are relatively common. In patients treated with vemurafenib on BRIM3, 38% required a dose reduction because of short-term side effects; 28% of patients treated with dabrafenib on BREAK3 required a dose reduction.[3,4] Most of these toxicities are tolerable and reversible. However, concern has arisen over an increase in proliferative events, most notably squamous cell carcinomas (SCCs), keratoacanthomas (KAs) and melanomas *de novo*.[7]

Paradoxical toxicities of selective BRAF inhibitors

Most targeted agents would be expected to have a suppressive effect (or null effect) on pathway signaling in cellular processes regardless of the genetic composition. A paradoxical effect has been observed with selective BRAF V600E mutant kinase inhibitors, where exposure to these drugs can lead to MAPK pathway activation in BRAF wild-type and low-activity BRAF-mutant cells.[7] The underlying mechanisms of paradoxical MAPK activation have been attributed to promotion of wild-type BRAF and CRAF dimerization and transactivation of the noninhibited RAF protein leading to subsequent MAPK pathway activation (Figure 1c). This process also appears to be dependent on upstream RAS signaling, such as through receptor tyrosine kinase activation and oncogenic RAS mutations. The paradoxical MAPK activation with selective BRAFi is believed to be involved in the proliferative events (paradoxical oncogenesis) seen during vemurafenib and dabrafenib treatment.

Cutaneous

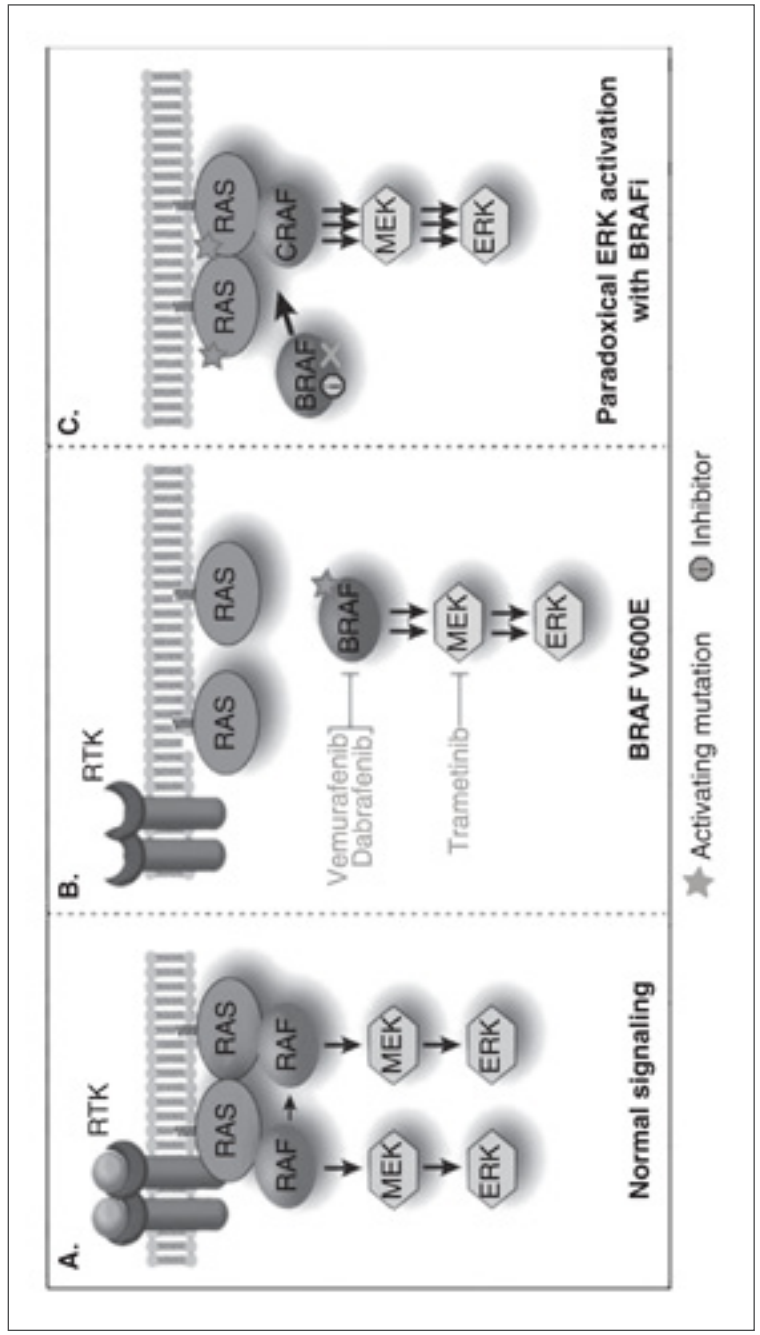
In the BRIM3 study of vemurafenib, 199 grade 2-3 cutaneous adverse events were reported in 336 patients.[3] Similarly, a high number of cutaneous side effects were reported in the BREAK3 study of dabrafenib (52 grade 2-3 cutaneous events in 187 patients).[4] While many of these toxicities included rash, alopecia, pruritus and hyperkeratosis, other more concerning proliferative toxicities were seen. With vemurafenib, SCCs and KAs occurred in 12 and 8% of patients, respectively.[3,8] With dabrafenib, SCCs or KAs occurred in 6% of patients.[4] Moreover, verrucal keratoses have been reported in up to 49% of patients on dabrafenib in an Australian series.[9] The vast majority of SCCs occur in chronically sun-damaged skin. Histologically, the SCCs tend to be well-differentiated lesions.[9] The mean time to diagnosis of the first cutaneous SCC/KA is 8-10 weeks, although lesions appear as early as three weeks.[9]

This short time lapse suggests that selective BRAFi may not have direct carcinogenic effects, but instead may potentiate preexisting initiating oncogenic events. In approximately 60% of cases, RAS mutations have been identified (predominantly HRAS).[8] Both SCCs and KAs can be treated by simple excision or cryotherapy. Occasionally, the distribution of these lesions can be quite extensive, but so far, no cases of metastases have been reported. Although less common, another proliferative skin disorder reported in patients on BRAFi is the occurrence of new melanocytic nevi and melanoma, commonly having a wild-type BRAF status.[9] The long-term consequences of these proliferative events remain unclear. The time to development of cutaneous lesions can be delayed as late as 25 weeks and tends to continue during the course of therapy.[10]

Gastrointestinal

Apart from diarrhea, nausea and vomiting, which are the most frequently reported side effects after cutaneous toxicities, the development of colonic and gastric polyps has been reported in patients receiving vemurafenib. In the phase 1 trial of vemurafenib, four out of eight long-term responders (>2 years) underwent endoscopic analysis; three of these patients

Figure 1 - Activation of the MAPK pathway



A. During normal signaling conditions, the MAPK cascade is initiated through ligand-mediated activation of receptor tyrosine kinases. In this model, the binding of ligand to its cognate receptor leads to recruitment of RAS to the plasma membrane, the formation of RAF dimers, and ultimately downstream activation of MEK and ERK. B. Acquisition of mutations in BRAF at codon position 600 (V600E) leads to constitutive activation of the MAPK pathway that is not dependent on upstream RTK or RAS activity. The kinase inhibitors vemurafenib and dabrafenib target the mutant form of BRAF, and trametinib targets MEK. C. In cells with a wild-type BRAF and either upstream growth-factor–activated or mutated RAS, the inhibitor binds BRAF and promotes BRAF-CRAF dimer formation leading to paradoxical activation of MAPK through transactivation of the uninhibited CRAF protomer

harbored multiple colonic adenomas and/or gastric polyps, an uncommonly high ratio.[11] One of these patients presented with a gastrointestinal bleed and was found to have 11 colonic and gastric polyps and a bleeding duodenal ulcer; he had an unrevealing endoscopy just five months before starting vemurafenib. The majority of the lesions sequenced harbored mutations in the APC tumor suppressor gene, which is known to be associated with sporadic and hereditary colorectal cancer. This is an unsettling finding since some evidence suggests that APC loss and MAPK signaling are required for the development of colorectal carcinoma in mouse models.[7]

Furthermore, a case of recurrent KRAS mutant colon cancer has been reported in a patient during treatment with dabrafenib plus trametinib therapy for metastatic BRAF-mutant melanoma.[12] Prior to his melanoma diagnosis, he underwent resection of localized colon cancer. His melanoma responded to BRAFi/MEKi therapy; however, after 12 weeks, an isolated brain lesion developed. After resection of this brain metastasis, pathology confirmed that it was a recurrence of his prior colon cancer. Cell lines derived from this KRAS mutant adenocarcinoma brain metastasis showed sensitivity to trametinib, whereas dabrafenib increased cell proliferation. After a temporary hold of drugs in this patient, single agent dabrafenib was restarted. Despite showing response in his melanoma disease, he experienced a rise in CEA levels, new pleural disease and a second brain metastasis confirmed to be colon adenocarcinoma.

Other proliferative disorders

The proliferative effects of paradoxical MAPK activation are not restricted to skin and gastrointestinal tract. The emergence of other types of malignancies has been described, such as RAS mutant leukemia, where vemurafenib was stimulating the growth of preexisting NRAS mutant chronic myelomonocytic leukemia cells by causing hyperactivation of ERK, after a mere 11 days of treatment.[7]

Expert opinion

The field of BRAF-targeted therapy is rapidly evolving. While the main goal is to increase clinical efficacy and duration of response, we will hopefully also see a reduction in paradoxical MAPK activation and secondary malignancies. One such strategy is the combination of BRAF and MEK inhibitors. The rationale is based on the reactivation of the MAPK pathway that occurs at time of BRAFi resistance. Indeed, the phase 1/2 study of dabrafenib plus trametinib in metastatic BRAF V600 mutant melanoma demonstrated a higher objective response rate and longer mPFS with the combination; a phase 3 study of dabrafenib plus trametinib versus dabrafenib plus placebo is ongoing.[13] The addition of MEKi also appears to reduce paradoxical MAPK activation, as the incidence of SCCs was 19% in the dabrafenib only cohort and 7% in the dabrafenib plus trametinib cohorts. However, the addition of MEKi can increase the risk of other side effects. MEKi are associated with peripheral edema, hypertension, decreased cardiac ejection fraction, and ocular events. Combination therapy does not fully prevent the development of secondary malignancies, but it does dramatically lower the prevalence of SCCs from 19% for dabrafenib alone to 2-7% in combination with trametinib.[6]

Perhaps the new generation of MAPK pathway inhibitors will overcome the paradoxical MAPK activation seen with selective BRAFi. These include RAF kinase inhibitors with more potent inhibition of all RAF isoforms, called paradox breakers. An example is the development of TAK-632, which suppresses RAF activity in BRAF wild-type cell with minimal paradoxical MAPK activation and has potent activity in BRAF-mutant melanoma cell lines.[14] ERK inhibitors are also being developed as single agents and in combination with BRAFi, which may also increase antitumor activity and eliminate paradoxical oncogenesis.[15]

As of yet, no trials have been conducted to specifically investigate the consequences of long-term BRAFi therapy. With emerging data on secondary cancers and more widespread use of BRAFi in patients with BRAF-mutant melanoma and other malignancies, this will be an important concept to address. While no firm guidelines exist, we recommend close follow-up by a dermatologist after commencing BRAF-targeted therapy. Since BRAFi treatment seems to provoke previous existing or dormant RAS mutant cancers, caution is warranted in the treatment of patients with a history of such malignancies. Once more data on the emergence of colonic and gastric polyps is available, the role of endoscopic screening can be better addressed. Identification of these paradoxical effects and toxicities will be necessary for the clinician to recognize and for future research development.

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part III

Developments in melanoma brain metastasis

8

Improved survival of patients with melanoma brain metastases in the era of targeted BRAF and immune checkpoint therapies

Abstract

Introduction
The development of brain metastases is common for systemic treatment failure in melanoma patients and has been associated with a poor prognosis. Recent advances with BRAF and immune checkpoint therapies have led to improved patient survival. We evaluated the risk of *de novo* brain metastases and survival of patients with melanoma brain metastases (MBM) since the introduction of more effective therapies.

Methods
Patients with unresectable stage III/IV melanoma who received first line systemic therapy at Moffitt Cancer Center between 2000 to 2012 were identified. Data was collected on patient characteristics, staging, systemic therapies, MBM status/management, and overall survival (OS). Risk of *de novo* MBM was calculated using a Generalized Estimating Equation model and survival comparisons were performed by Kaplan-Meier and Cox proportionate analyses.

Results
610 patients were included, of which 243 were diagnosed with MBM (40%). MBM patients were younger with a lower frequency of regional metastasis. No significant differences were noted in gender, BRAF status or therapeutic class. The risk of *de novo* MBM was similar among chemotherapy, biochemotherapy, BRAF-targeted therapy, ipilimumab and anti-PD1/PD-L1 regimens. MBM patient median OS was significantly shorter when determined from time of first regional/distant metastasis but not from time of first systemic therapy. Median OS from time of MBM diagnosis was 7.2, 8.5 and 22.7 months for patients diagnosed 2000-2008, 2009-2010, and 2011-present, respectively (p = 0.002).

Conclusion
Brain metastases remain a common source of systemic treatment failure. OS of MBM patients has significantly improved. Further research into MBM prevention is needed.

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Introduction

More than one-third of patients with advanced melanoma will develop brain metastases during the course of their disease, and even higher rates have been observed on autopsy.[1-3] Historically, the prognosis of patients with melanoma brain metastases (MBM) has been poor, with median overall survival (OS) ranging from 3-6 months from time of diagnosis.[1,2,4,5] Patients with solitary or oligometastatic disease amenable to surgery or stereotactic radiosurgery (SRS) have better survival with median OS reported from 7-10 months.[6] There is no method to accurately predict who will develop MBM. However, various parameters are associated with an increased risk, e.g. melanoma arising from head and neck areas, ulcerated primaries, elevated serum lactate dehydrogenase levels and possibly molecular alterations in BRAF, NRAS or PTEN.[2,5,7,8]

The brain has been a prominent site of treatment failure with systemic therapies for advanced melanoma patients. In a prospective study evaluating MBM incidence between cisplatin/temozolomide/IL-2 and cisplatin/dacarbazine/IL-2, 49% of assessable melanoma patients developed CNS disease with no significant difference between treatments.[9] Similarly, MBM progression has been reported as a primary relapse site in up to half of patients who initially responded to IL-2.[10] These observations may be due to historically low rates of controlling systemic disease (i.e. prevention of tumor seeding to the brain), as well as poor CNS penetration and MBM activity of many systemic therapies.[11] Among chemotherapies with modest CNS activity, e.g. temozolomide and fotemustine, studies have shown objective MBM response rates ranging from 7-12%.[12] Similar disappointing results were seen in patients treated with high-dose IL-2.[13,14]

New immune checkpoint and BRAF-/MEK-targeted therapies have demonstrated greater clinical activity in metastatic melanoma patients. Median OS has now reached two years and longer in studies of BRAF/MEK combination therapy and anti-PD-1 regimens.[15-17] Phase 2 trials of these agents in patients with active MBM have also demonstrated promising intracranial activity with objective MBM response rates as high as 22% with pembrolizumab and 31% with dabrafenib (BRAF V600E mutant population).[18-21] While these findings suggest that improved melanoma patient outcomes could be in part due to a reduction in CNS failure with enhanced extracranial disease control and/or CNS activity, the brain has been reported to still be a common site of treatment failure for BRAF-targeted therapy.[22,23] Therefore, it remains unclear if MBM incidence rates significantly differ among newer targeted and immune therapies compared to prior treatment strategies and if patient survival continues to be significantly impacted by the development of MBM.

The primary objective of this study was to investigate the association between systemic therapy regimens and *de novo* MBM development in advanced melanoma patients treated with chemotherapy, biochemotherapy, interleukin-2, BRAF-targeted agents, or immune checkpoint blockade. The secondary objectives were to compare the overall survival (OS) in advanced melanoma patients with and without brain metastases and assess prognostic factors in MBM patients treated with new targeted and immune therapy strategies.

Materials and methods

This was a retrospective cohort study of patients with unresectable metastatic melanoma (cutaneous/unknown primary, uveal, or mucosal origin) treated with systemic therapy at Moffitt Cancer Center. To include a comprehensive sample size, patients were identified using a combination of pharmacy treatment records, BRAF genotyping records and clinical trial enrollment. Inclusion requirements were stage III or IV melanoma, initiation of systemic therapy between 2000-2012 to allow for long-term follow-up and at least two months of follow-up on first line systemic therapy. Data were collected on patient demographics, clinical/pathologic data on the primary melanoma and subsequent metastases, systemic therapy treatment, and OS. Patients with unknown primaries were added to the cutaneous group based on recent literature unless there was a suspicion by the treating investigator that the tumor was not cutaneous in origin.[24,25] Patients were then divided in three groups (2000-2008; 2009-2010 and from 2011 onwards), based on the introduction of targeted therapies. In 2009-2010, an increasing number of checkpoint/targeted therapy trials became available and 2011 was the approval year for ipilimumab and vemurafenib. This also divided the patients into roughly equal groups for statistical analyses.

Because of the range of systemic therapies that patients received - both standard therapies and clinical trial agents - seven categories were utilized to represent generalized treatment approaches available in clinical practice. These include chemotherapy regimens (monotherapy and combinations), biochemotherapy regimens (E3695 regimen or similar)[26], IL-2, ipilimumab (allowed for combined ipilimumab plus other non-checkpoint immunomodulators such as interferon), anti-PD-1/PD-L1 therapies (e.g. pembrolizumab and nivolumab as monotherapy or in combination with other non-immune checkpoint stimuli such as a multipeptide vaccine), and BRAF-targeted therapy (selective BRAF inhibitor monotherapy, MEK inhibitor monotherapy, and combination BRAF plus MEK inhibitors). The remaining group ('Other') contained all regimens that did not fit exclusively into one of these categories (e.g. dendritic cell vaccines, combination regimens on protocol such as carboplatin/paclitaxel/sorafenib and ipilimumab/vemurafenib. This group also contained a patient on ipilimumab/nivolumab).The study was approved by the Institutional Review Board of the University of South Florida. MBM patients were defined as patients who developed MBM at any time during follow-up, regardless of preceding and subsequent treatment. Patients classified as developing MBM prior to starting systemic treatment were classified as being diagnosed before the initiation date of first systemic therapy. MBM patients never receiving systemic treatment during the course of their disease were not captured.

Descriptive statistics were summarized for age, gender, primary melanoma type, BRAF status and systemic therapy received for all patients and classified by MBM status. The first set of analyses focused on assessing the association between variables of interest related to MBM development. Clinical and demographic characteristics between MBM and MBM-free populations were compared. Proportion differences between the two populations were investigated using Chi-square tests for categorical variables. Monte Carlo estimated *p*-values for the exact test were reported when ≥50% of the cells have expected counts less than 5. Median differences between MBM and MBM-free populations for continuous variables (e.g. age) were compared using Wilcoxon rank-sum tests. We then evaluated the association between treatment (coded as the seven categories of therapy as described above), systemic treatment line (first, second and third line of therapies only), age at first systemic treatment, and the development of MBM using a Generalized Estimating Equation (GEE) model. Since patients often received more than one line of systemic therapy, the GEE model was performed in order to evaluate the correlation between each line of therapy and MBM event in the same patient. Patients with recurring MBM were censored for subsequent therapies. For example, a patient who was MBM-free during ipilimumab as first line therapy, but then developed MBM during second line therapy with a BRAF inhibitor would have been classified as a negative event followed by a positive event. The third line therapy of this patient would not have been included in the model.

OS, defined as the duration between first diagnosis of regional or distant metastatic disease to date of death, was evaluated in both MBM and MBM-free patients using the Kaplan-Meier (KM) method. Survival differences between the two populations were determined using a Logrank test. This survival analysis was repeated using time zero as date of first systemic therapy. Subsequent survival analyses were focused on MBM patient survival, which were calculated from date of MBM diagnosis to date of death. KM method, as well as univariate and multivariate Cox proportional-hazards regression models, were used to determine whether variables were associated with OS and to obtain hazard ratios (HR) and their confidence intervals. All statistical analyses were performed using SAS (version 9.4, SAS Institute Inc., Cary, NC). A two-sided *p*-value ≤0.05 was considered statistically significant.

Results

Patient Characteristics

1016 Patients were initially evaluated for inclusion in this data set. Patients were excluded because of <2 months follow-up after start of systemic treatment (n = 245), no digital records

available (n = 116), non-melanoma cancer diagnosis (n = 40), multiple melanoma primaries (which confounded start dates; n = 4) and missing date of diagnosis (n = 1). 610 patients were included in the database (Supplement Figure A). Median follow-up was 27.6 months from time of first regional or distant metastasis.

Of the 610 patients included in the data set, 243 patients (39.8%) developed MBM. The median time from initial melanoma diagnosis to first MBM diagnosis was 29.6 months (range 0-320.2 months). MBM patients were significantly younger than non-MBM patients at date of first metastasis (median 58 versus 62 years, $p \leq 0.0001$; Table 1). There was a significant difference in the primary melanoma subtypes between MBM and MBM-free populations ($p = 0.02$; Table 1), largely driven by the low number of MBM patients with a mucosal primary site ($p = 0.008$). Also, patients in the MBM population were less likely to have regional metastasis (stage III) as the first site of metastasis ($p < 0.0001$). Otherwise, there were no significant differences in gender, BRAF-status or class of systemic treatments received between the MBM and MBM-free populations.

The first MBM event was most often diagnosed early in the disease, i.e. prior to systemic therapy (31.7%) or during first-line treatment (35.4%) as shown in Supplement Table A. Neurologic symptoms were present in 53.5% of patients at the time of MBM diagnosis. Karnofsky performance status (KPS) was >70% in 59.7% of patients. Most patients (48.1%) had 1 MBM at diagnosis, 34.6% had 2-4 MBM and 16.5% had 5 or more MBM and/or leptomeningeal disease. Most frequent primary MBM treatment was SRS in 118 patients (48.6%), followed by WBRT in 38 patients (15.6%), craniotomy in 37 patients (15.2%), start of new systemic treatment in 3 (1.2%) and continuation of prior systemic treatment in 2 (1.0%). 13 patients (5.4%) received no treatment for MBM. The remainder of patients received combination treatments such as SRS plus WBRT.

Development of *de novo* MBMs during the administration of systemic therapy

The association between patient age, line of systemic therapy, or class of systemic therapy (first through third line only) and the *de novo* MBM incidence rates were investigated using a GEE model to account for multiple lines of treatment received by the same patient. Patients with recurring MBM were censored for subsequent therapies. While there was a trend for association between age and risk of developing *de novo* MBM ($p = 0.08$), no association was demonstrated between line of therapy or class of systemic therapy and the risk of developing *de novo* MBM ($p = 0.68$ and 0.85, respectively). With regards to the latter using chemotherapy as the reference group, odds ratios for developing *de novo* MBM were 1.5 (95%CI: 0.70-3.02) with biochemotherapy, 1.1 (95%CI: 0.60-1.99) with ipilimumab, 1.0 (95%CI: 0.40-2.83) with anti-PD-1/anti-PD-L1, and 1.3 (95%CI: 0.60-2.49) with BRAF-targeted therapy (Figure 1).

Table 1 - Patient Characteristics

Characteristic	Overall (n = 610)	MBM population (n = 243)	MBM-free population (n = 367)	*p*-value
Age* (median, range)	60 (15-92)	58 (15-86)	62 (19-92)	<0.0001
Gender (male)	400 (65.6%)	159 (65.4%)	241 (65.7%)	1.0
Primary melanoma type				0.02
Cutaneous	583 (95.6%)	239 (98.4%)	344 (93.7%)	
Mucosal	19 (3.1%)	2 (0.8%)	17 (4.6%)	
Ocular	6 (1.0%)	2 (0.8%)	4 (1.1%)	
Other	2 (0.3%)	0 (0.0%)	2 (0.5%)	
Stage at first metastasis				<0.0001
Stage III	274 (44.9%)	82 (33.7%)	190 (51.8%)	
Stage IV	336 (55.1%)	161 (66.3%)	177 (48.2%)	
BRAF status				0.4
BRAF V600 mutant	120 (19.7%)	54 (22.2%)	66 (18.0%)	
BRAF V600 wild-type	159 (26.1%)	61 (25.1%)	98 (26.7%)	
Unknown	331 (54.3%)	128 (52.7%)	203 (55.3%)	
Class of systemic therapies**				
BRAF pathway inhibitor	90 (14.8%)	39 (16.0%)	51 (13.9%)	0.5
Interleukin-2	80 (13.1%)	35 (14.4%)	45 (12.3%)	0.5
Anti-CTLA-4	188(30.8%)	77 (31.7%)	111 (30.2%)	0.7
Anti-PD-1/PD-L1 therapy	50 (8.2%)	20 (8.2%)	30 (8.2%)	1.0

** Age at date of first regional or distant metastasis*
*** Only 1st through 3rd line therapies*
MBM: melanoma brain metastases

Overall Survival

OS, defined as the duration from date of first metastasis to death, was evaluated by KM analysis for all patients (Figure 2a). The median OS of all patients was 30.9 months (95% CI: 28.2-36.4). Survival probabilities at one year, two years and three years were 79.7% (95% CI: 76.3- 82.8%), 60.6% (95% CI: 56.3-64.6%) and 45.9% (95% CI: 41.4-50.3%), respectively. OS was significantly different between MBM and MBM-free patient groups (median OS 25.9 months and 35.5 months, respectively, p = 0.048; Figure 2b). The three year OS rates were 40.2% (95% CI: 33.3-47.0%) for MBM patients and 49.8% (95% CI: 43.9-55.5%) for MBM-free patients. Because fewer patients with MBM diagnosis had regional disease as the first metastasis, OS was also evaluated from start of first systemic therapy to death for further characterization (Supplement Figure B). Median OS from date of first systemic therapy was 20.3 months (95% CI: 16.9-24.9 months) for MBM-free patients and 14.7 months (95% CI: 13.0-21.5 months) for MBM patients (p = 0.1755).

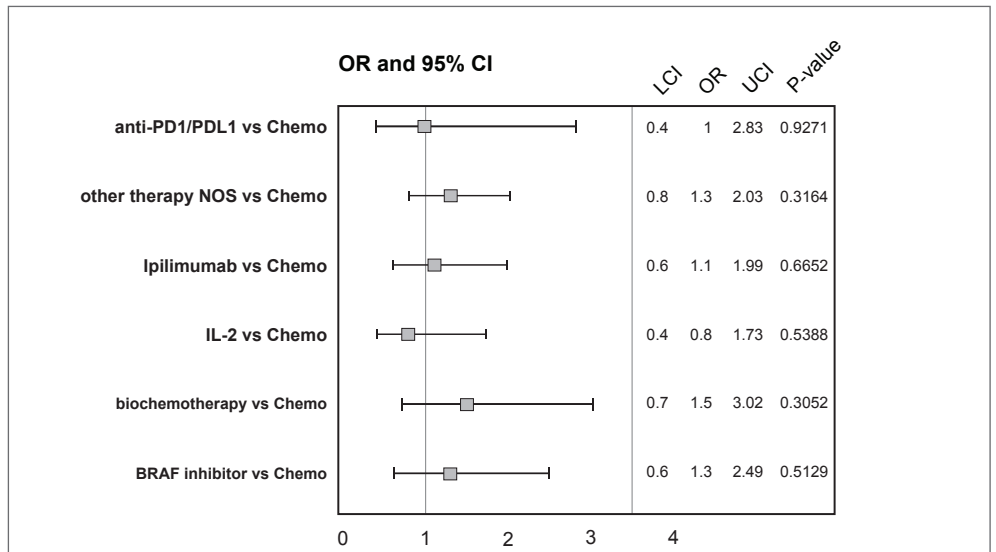
Data was then analyzed separately in the MBM cohort. Median OS from date of MBM diagnosis to date of death was 10.5 months (95% CI: 8.6-12.8 months; Figure 3A). Survival probabilities at one, two and three years were 43.4% (95% CI: 36.6-50.1), 27.3% (95% CI 20.5-34.4%) and 17.5% (95% CI: 11.3-24.9%), respectively.

Prognostic factors for MBM patients

Variables previously identified to be associated with MBM prognosis were evaluated by KM analysis (using survival from date of MBM diagnosis to death). These included age, gender, BRAF V600 mutation status, MBM number, neurologic symptoms, KPS, Diagnosis-Specific Graded Prognostic Assessment (DS-GPA) and primary type of MBM management (Supplement Table A). MBM year diagnosed and line of therapy when first MBM developed were included in the analysis as well. Of these factors, longer OS was associated with a later year of MBM diagnosis (2011-present), fewer MBM (1 or 2-4 MBMs), absence of neurologic symptoms, primary MBM treatment (SRS or craniotomy), and better KPS/DS-GPA scores (Supplement Table B; all p<0.05). In particular, median OS was 22.7 months in patients who were diagnosed with MBM in 2011 or later, as compared to 8.5 months and 7.5 months for patients diagnosed with MBM between 2009-2010 and 2000-2008, respectively, (p = 0.0002; Figure 3B).

Similar findings were observed using a univariate Cox model to study variables associated with risk of death in MBM patients (Supplement Table C). Statistically significant variables (MBM year of diagnosis, MBM number, neurologic symptoms, KPS, and primary MBM treatment) were then analyzed using a multivariate Cox model (Table 2). DS-GPA was not included as it incorporates both MBM number and KPS. All variables showed statistically significant independent associations with risk of death. Risk of death was 2.8 and 2.0 fold greater for patients diagnosed with MBM between 2009-2010 or 2000-2008, respectively, when compared to those diagnosed between 2011-present. Hazard ratios for risk of death in patients with 2-4 MBM and ≥5 MBM and/or leptomeningeal disease were 1.5 and 2.0,

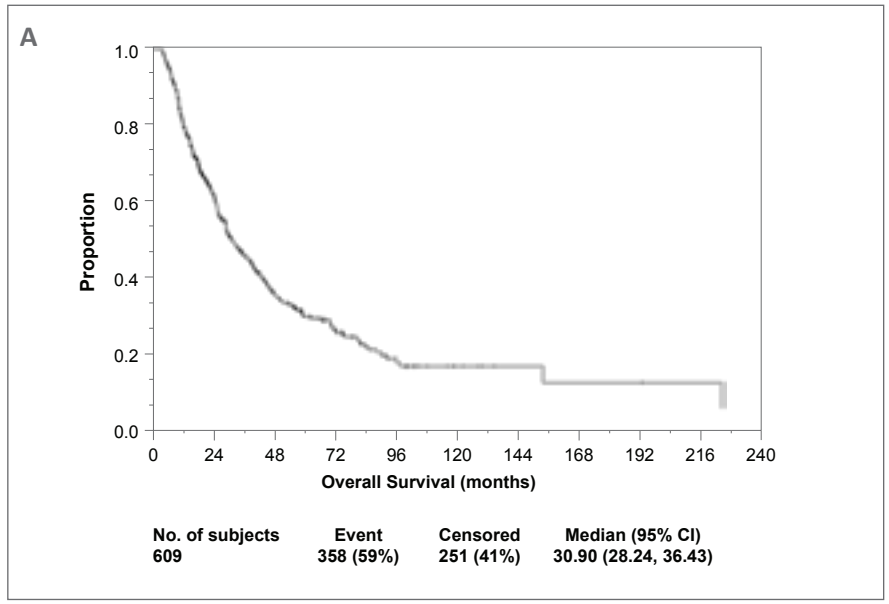
Figure 1 - Forest plot of odds risk of developing *de novo* melanoma brain metastases (MBM) during systemic therapy

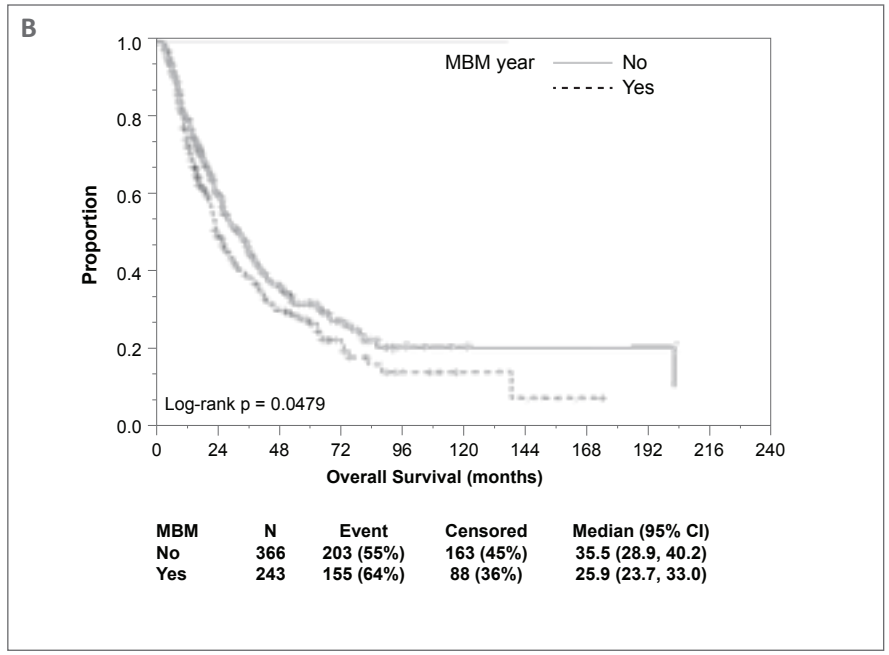


Odds ratio (OR) for developing de novo MBM with each class of therapy was determined using chemo-therapy as the denominator. 95% confidence interval intervals reported
LCI: lower confidence interval; UCI: upper confidence interval; NOS: not otherwise specified

respectively, compared to 1 MBM. Patients with neurologic symptoms had a HR of 2.0 in comparison to asymptomatic MBM patients. While a KPS of <70 had a HR of 2.4 in comparison to KPS >90- 100, there was no significant difference between a KPS of 70-90 and >90-100. Receipt of BRAF-targeted therapy and/or immune checkpoint therapy was analyzed to determine association with MBM year of diagnosis for contribution to improved OS. The majority of patients (72%) who received one or more of these therapies were diagnosed with the first MBM in 2011 or after (chi-square 92.13, <0.0001). Furthermore, receipt of BRAF-targeted therapy and/or immune checkpoint therapy was associated with improved OS using a multivariate cox model (Supplement Table D). However, the significance was diminished when both MBM year of diagnosis and type of therapy received were included in the model (data not shown).

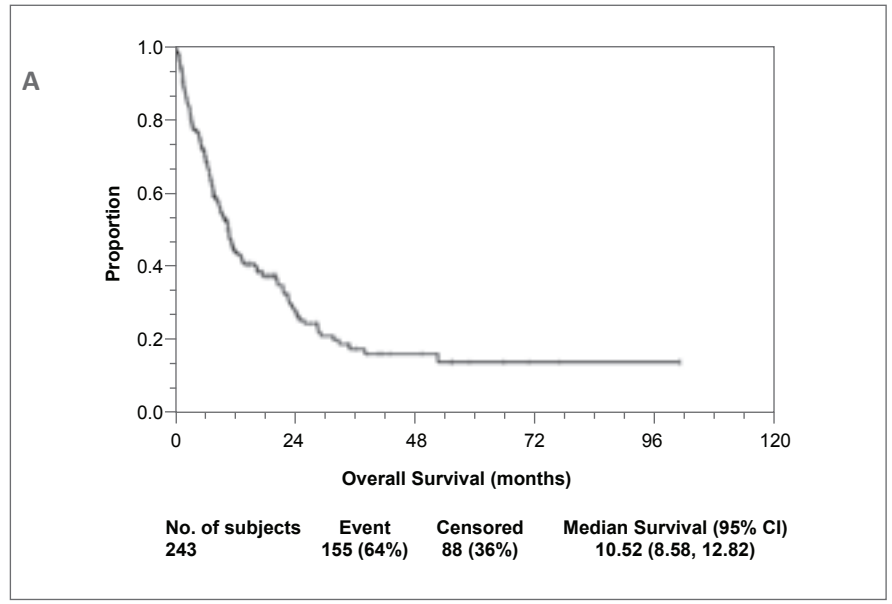
Figure 2 - Overall survival (OS) from the date of first regional or distant metastasis to death

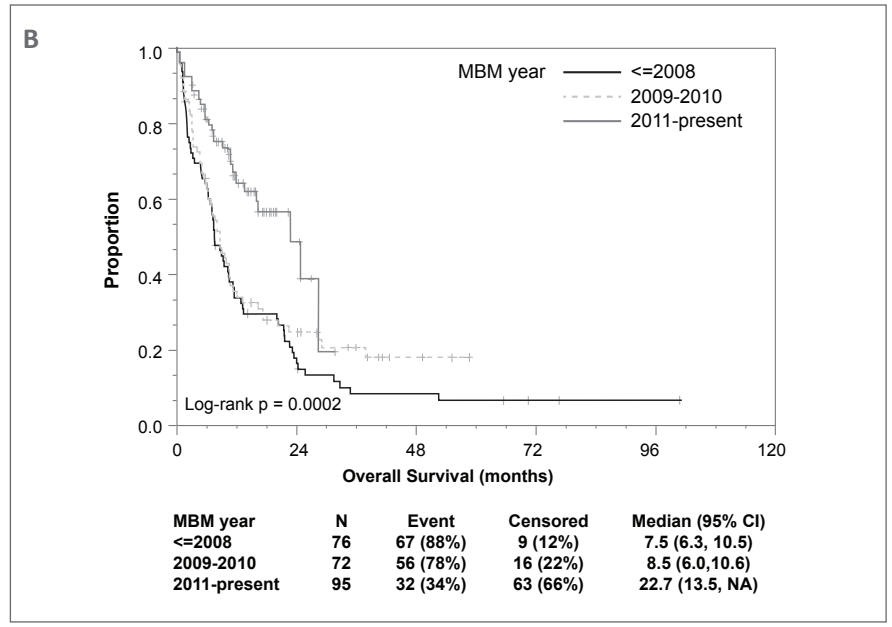




The OS for all patients is shown (A), as well as the OS for patients diagnosed with melanoma brain metastasis (MBM) diagnosis compared to those with no MBM diagnosis during the course of disease (B). Tick marks represent patient censoring
CI: confidence interval

Figure 3 - Overall survival (OS) of melanoma brain metastases (MBM) patients from date of first MBM diagnosis to death





The OS for all MBM patients is shown (A). Survival at 12, 24, and 36 months was 43.4% (95% CI: 36.6, 50.1%), 27.3% (95% CI: 20.5, 34.4%), and 17.5% (95% CI: 11.3, 24.9%), respectively. OS for MBM patients by year group of MBM diagnosis is shown (B)
CI: confidence interval

Table 2 - Multivariate cox model for melanoma brain metastases (MBM) prognostic factors

Parameter	Comparison	Hazard Ratio (95% CI)	*p*-value
MBM year	</=2008 vs. 2011-present	1.98(1.10, 3.56)	0.0226
	2009-2010 vs. 2011-present	2.77(1.58, 4.87)	0.0004
Number of MBM	2-4 vs. 1	1.52(0.92, 2.52)	0.1038
	>/=5 or leptomeningeal vs. 1	1.95(1.04, 3.66)	0.0374
Neurologic symptoms	Yes vs. no	1.95(1.16, 3.30)	0.0123
MBM line of systemic therapy	1/1-2 vs. 0 (before systemic therapy)	1.20(0.71, 2.04)	0.4999
	2/2-3 vs. 0 (before systemic therapy)	4.72(2.55, 8.72)	<.0001
	>/=3 vs. 0 (before systemic therapy)	1.64(0.86, 3.12)	0.131
MBM primary treatment	None vs. SRS	2.66(1.13, 6.25)	0.0254
	Surgery vs. SRS	1.50(0.77, 2.94)	0.2312
	WBRT vs. SRS	0.98(0.54, 1.75)	0.9309
KPS	</=70 vs. >90-100	2.41(1.19, 4.86)	0.0142
	>70-90 vs. >90-100	1.08(0.65, 1.78)	0.7708

KPS: Karnofsky Performance Status; SRS: stereotactic radiosurgery; WBRT: whole brain radiation therapy

Discussion

In this retrospective study, MBM incidence and MBM patient survival were investigated and compared to outcomes of advanced stage patients without MBM. To the best of our knowledge, this resulted in one of the largest MBM cohort reported to date with inclusion of patients receiving approved BRAF-targeted and immune checkpoint therapy. The following key observations were made: (1) the overall incidence of *de novo* MBM in patients with advanced melanoma receiving systemic therapy was 40%, which primarily occurred prior to or during the first line of therapy; (2) the incidence of MBM was not significantly different with BRAF-targeted agents, ipilimumab or anti-PD-1/PD-L1 therapy compared to traditional chemotherapy; (3) the median OS of MBM patients was statistically shorter than MBM-free patients from time of first regional or distant metastasis but not from start of first line systemic therapy; and (4) the median OS of MBM patients was significantly longer in patients diagnosed with MBM in 2011 or after, which was independent of other MBM prognostic factors.

The MBM incidence rate in our study is consistent with past studies where 44% of melanoma patients with unresectable stage III/IV disease developed MBM.[1,27] Also, the lack of an association between BRAF status and MBM incidence was similar to several prior retrospective studies.[5,8,27] However, BRAF status was unknown in 37% of patients in our study, due to BRAF testing not being routinely conducted before the approval of vemurafenib in 2011, which may have impacted results. With regards to the timing of *de novo* MBM, patients were most likely to be diagnosed prior to or during the first line of systemic therapy (27% of all patients). This supports NCCN recommendations for inclusion of brain imaging for initial staging and monitoring of patients with advanced melanoma.[28] The fact that patients were still diagnosed frequently with *de novo* MBM during second line therapy and after also supports the need for continued surveillance in patients undergoing therapy; however, the frequency with which to screen for MBM is not well defined.

Contrary to what may have been expected, the rate of *de novo* MBM was not significantly lower in patients treated with newer targeted and immunotherapy agents that demonstrate objective CNS anti-tumor activity.[18-21] For selective BRAF inhibitors, limited drug penetration across the blood brain barrier and possible brain derived factors produced from astrocytes that enhance tumor survival may be contributing factors.[29,30] In a similar fashion, the CNS has been described as an immune privileged site where direct stimulation or recruitment of cytotoxic T cell populations may be less robust compared to extracranial tumor sites with immunotherapy.[31] Another possibility is that neither class of therapies directly target the biology underlying brain tropism for some melanoma tumors.[7,32]

Encouragingly, the median OS of patients with MBM in our data set appears much improved compared to historical data. Davies et al. reported a median OS of 4.7 months after MBM diagnosis in who developed MBM during clinical trial participation between 1986-2004.[1] In our study, median OS was 10.5 months from time of MBM diagnosis for the entire MBM patient population, which was largely driven by substantially improved survival seen in patients diagnosed in 2011 or after (median 22.7 months for this patient population). Our results are supported by multiple smaller retrospective studies where median MBM patient survival with SRS and either BRAF therapies or immune checkpoint therapy has been one to two years.[33-37] More importantly, the gap in OS between patients with and without MBM appears to be narrowing and was not statistically significant in our study when determined from time of first systemic therapy.[34,36,38-44]

Several limitations exist in the current study. By identifying patients largely based on systemic therapy records, MBM patients who never received systemic treatment due to cure by craniotomy or SRS or death prior to therapy were not captured. Exclusion of patients with less than two months follow-up on systemic therapy might have added to this latter bias, causing an over-estimation of OS. However, this type of bias is present in other published studies (e.g. Davies et al).[1] Another limitation is the potential variability of surveillance brain imaging. Many of the patients receiving BRAF-targeted and immune checkpoint therapies participated in clinical trials where brain imaging was routinely performed and could have introduced a lead time bias. Inevitably, bias arises from separating treatments out by line. Current cancer care has become increasingly complex and many MBM patients receive a combination of therapies, both brain directed therapies such as WBRT/SRS and systemic therapies. Lastly, the focus of this study was on *de novo* MBM development during systemic therapy. Tracking progression in treated MBM and the development of subsequent MBM was beyond the scope of this investigation.

In conclusion, the development of brain metastases remains a clinical problem despite better OS in patients diagnosed since the introduction of BRAF-targeted and immune checkpoint therapies. This is in part reflective of the major advances in treating extracranial disease and more effective localized MBM control with craniotomy and SRS. Exclusion of patients with treated MBM from clinical trials is not appropriate given the more favorable survival of MBM patients. Future research on strategies to abrogate MBM development is warranted.

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Table A - MBM characteristics for Univariate and Multivariate analys

Characteristics	Variables	Subject number (%)
Age	< 65	185 (76.1%)
	>/= 65	58 (23.9%)
Gender	Female	84 (34.6%)
	Male	159 (65.4%)
BRAF V600 status	Mutant (V600E)	41 (16.8%)
	Mutant (other)	13 (5.3%)
	Wild-type	61 (25.1%)
	Not tested/Unknown	128 (52.7%)
MBM year diagnosed*	</= 2008	76 (31.3%)
	2009 - 2010	72 (29.6%)
	2011 - present	95 (39.1%)
Line of therapy for first MBM	Prior to first therapy	77 (31.7%)
	First line/before second line	86 (35.4%)
	Second line/before third line	43 (17.7%)
	Third line or after	37 (15.2%)
MBM number	1	117 (48.1%)
	2-4	84 (34.6%)
	>5 or leptomeningeal disease	40 (16.5%)
	Unknown	2 (0.8%)
Neurologic symptomatic	Yes	108 (44.4%)
	No	130 (53.5%)
	Unknown	5 (2.1%)
KPS	</= 70%	24 (9.9%)
	>70% - 90%	95 (39.3%)
	>90% - 100%	50 (20.7%)
	Not reported	73 (30.2%)

Table A - continued

Characteristics	Variables	Subject number (%)
DS-GPA score	0 - 1	19 (7.8%)
	2	33 (13.6%)
	3	61 (25.2%)
	4	56 (23.1%)
	Unknown	73 (30.2%)
Type of MBM management**	SRS	118 (48.6%)
	WBRT	58 (23.9%)
	Surgery	40 (16.5%)
	Systemic therapy	3 (1.2%)
	Other	4 (1.6%)
	None	13 (5.3%)
	Unknown	7 (2.9%)

SRS = stereotactic radiosurgery; WBRT = whole brain radiation therapy
**Date ranges chosen based on relative availability of systemic therapies and relatively equal distribution of groups*
***Primary modality used to manage dominant active CNS disease at presentation*

Table B - Prognostic factors for Kaplan Meier overall survival from time of MBM diagnosis

Variable	Level	Total	Failed	Percentage Censored (%)	Median in years (95% CI)	Log-rank test *p*-value
Overall		242	154	36.36	0.9(0.7, 1.1)	.
Gender	Female	84	51	39.29	1.1(0.8, 1.9)	0.3473
	Male	158	103	34.81	0.8(0.6, 1.0)	.
Age	<65	185	120	35.14	0.9(0.7, 1.1)	0.4865
	>/=65	57	34	40.35	0.9(0.4, 1.3)	.
MBM year	</=2008	75	66	12.00	0.6(0.5, 0.9)	0.0002
	2009-2010	72	56	22.22	0.7(0.5, 0.9)	.
	2011-present	95	32	66.32	1.9(1.1, NE*)	.
Number of MBM	1	116	67	42.24	1.4(0.9, 1.9)	<.0001
	2-4	84	56	33.33	0.8(0.6, 1.0)	.
	>/=5 or leptomeningeal	40	30	25.00	0.5(0.2, 0.7)	.
Neurologic symptoms	No	130	73	43.85	1.0(0.9, 1.8)	0.0010
	Yes	107	78	27.10	0.6(0.4, 0.8)	.
MBM line of therapy	0 (before therapy)	77	47	38.96	1.7(0.9, 2.0)	<.0001
	1 & between 1-2	85	49	42.35	0.7(0.6, 1.7)	.
	2 & between 2-3	43	31	27.91	0.4(0.2, 0.9)	.
	>/=3	37	27	27.03	0.6(0.2, 0.9)	.
MBM primary treatment	None	13	11	15.38	0.2(0.0, 0.4)	<.0001
	Surgery	40	25	37.50	1.0(0.6, 1.9)	.
	SRS	118	72	38.98	0.9(0.8, 1.4)	.
	WBRT	58	41	29.31	0.6(0.4, 1.3)	.
BRAF status	Negative	61	24	60.66	1.9(1.1, NE*)	0.6644
	Positive	54	19	64.81	2.4(1.3, 4.4)	.
KPS	</=70	24	19	20.83	0.4(0.2, 0.7)	0.0006
	>70-90	95	51	46.32	0.9(0.6, 1.7)	.
	>90-100	50	36	28.00	0.9(0.6, 1.4)	.
DS-GPA	0-1	19	14	26.32	0.7(0.1, 1.3)	0.0083
	2	33	22	33.33	0.6(0.4, 0.7)	.
	3	61	38	37.70	0.8(0.6, 1.0)	.
	4	56	32	42.86	1.1(0.9, 2.4)	.

KPS = Karnofsky Performance Status; DS-GPA = Disease Specific-Graded Prognostic Assessment
**The upper limit of 95% CI could not be reliably estimated*

Table C - Univariate Cox model for MBM patients

Covariate	N	Comparison	Hazard Ratio (95%CI)	*p*-value
Gender	243	Male vs. Female	1.18(0.84, 1.65)	0.3370
Age	243	>/=65 vs. <65	1.15(0.79, 1.69)	0.4571
MBM year	243	</=2008 vs. 2011-present	2.38(1.55, 3.63)	0.0003
	.	2009-2010 vs. 2011-present	1.96(1.26, 3.04)	.
Number of MBM	241	2-4 vs. 1	1.46(1.02, 2.08)	<.0001
	.	>/=5 or leptomeningeal vs. 1	2.79(1.80, 4.32)	.
Neurologic symptoms	238	Yes vs. No	1.71(1.24, 2.35)	0.0010
MBM line of systemic therapy	243	1/1-2 vs. 0 (before systemic therapy)	1.45(0.97, 2.15)	<.0001
	.	2/2-3 vs. 0 (before systemic therapy)	2.58(1.63, 4.07)	.
	.	>/=3 vs. 0 (before systemic therapy)	2.32(1.44, 3.73)	.
MBM primary treatment	229	None vs. SRS	4.13(2.17, 7.86)	0.0001
	.	Surgery vs. SRS	1.08(0.68,1.70)	.
	.	WBRT vs. SRS	1.47(1.00,2.16)	.
BRAF status	115	Negative vs. Positive	1.15(0.62, 2.11)	0.6646
KPS	169	</=70 vs. >90-100	2.65(1.49, 4.70)	0.0011
	.	>70-90 vs. >90-100	1.02(0.66,1.57)	.
DS-GPA	169	0-1 vs. 4	2.38(1.26,4.48)	0.0103
	.	2 vs. 4	2.24(1.28, 3.91)	.
	.	3 vs. 4	1.44(0.90, 2.31)	.

Table D - Multivariate Cox model for MBM patients

Parameter	Comparison	Hazard Ratio (95% CI)	*p*-value
Type of Therapy	BRAFi/no-ICT vs. no-BRAFi/no-ICT	0.59(0.27, 1.30)	0.1895
	ICT/No-BRAFi vs. no-BRAFi/no-ICT	0.44(0.26, 0.74)	0.0022
	ICT/BRAFi vs. no-BRAFi/no-ICT	0.18(0.04, 0.77)	0.0209
Number of MBM	2-4 vs. 1	1.47(0.89, 2.41)	0.1307
	>/=5 or leptomeningeal vs. 1	2.01(1.06, 3.79)	0.0319
Neurologic symptoms	Yes vs. No	1.73(1.07, 2.82)	0.0269
MBM line of systemic therapy	1/1-2 vs. 0 (before systemic therapy)	1.31(0.77, 2.23)	0.3131
	2/2-3 vs. 0 (before systemic therapy)	4.86(2.62, 9.02)	<.0001
	>/=3 vs. 0 (before systemic therapy)	1.48(0.77, 2.83)	0.2415
MBM primary treatment	None vs. SRS	3.50(1.50, 8.20)	0.0038
	Surgery vs. SRS	1.35(0.70, 2.59)	0.3757
	WBRT vs. SRS	0.92(0.51, 1.67)	0.7800
KPS	</=70 vs. >90-100	2.19(1.11, 4.32)	0.0236
	>70-90 vs. >90-100	1.08(0.66, 1.77)	0.7614

BRAFi = Selective BRAF and/or MEK inhibitor; ICT = Immune checkpoint therapy

Figure A - Flow chart of patient inclusion

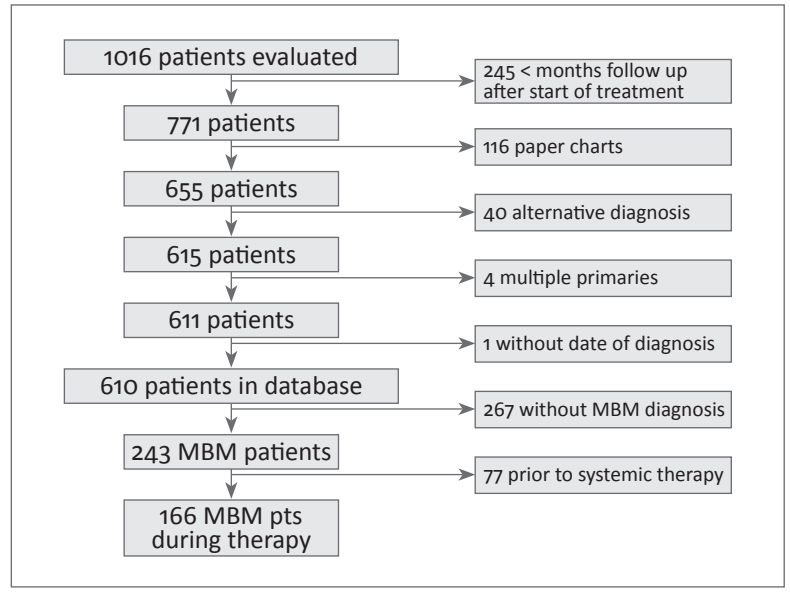
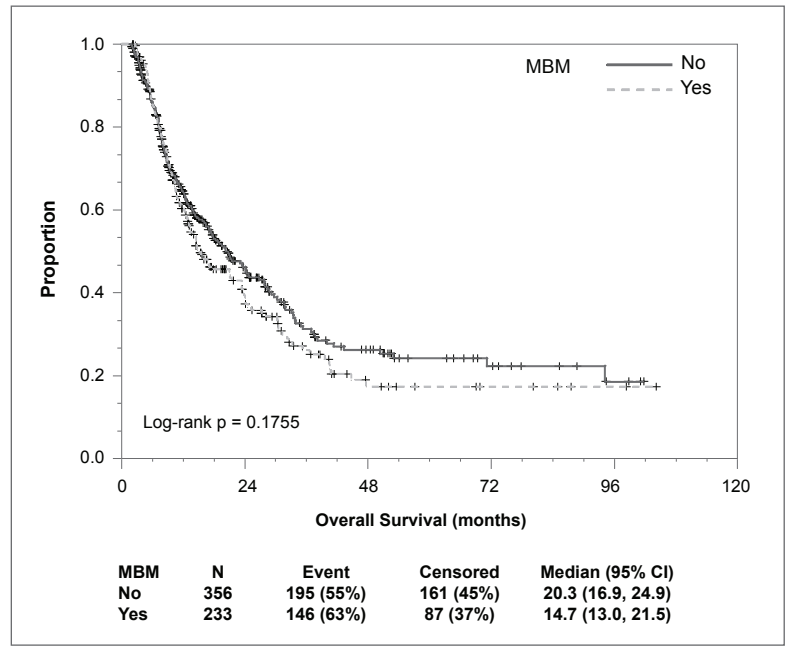


Figure B - Overall survival from the time of initiation of first line systemic therapy to death in patients with MBM and without MBM



9 Summary

English summary

An extensive introduction of melanoma and current treatment paradigms is given in **Chapter 1**, focusing on cutaneous melanoma, the most prevalent subtype of this tumor. This includes a brief history of melanoma and information on epidemiology, prognosis, clinical presentation, staging, pathology, molecular biology and current treatments. A separate section details information on prevalence and treatment of melanoma brain metastasis, one of the biggest clinical challenges today and an area of unmet need, with a historical prognosis of only 2-5 months from time of diagnosis and an area of unmet medical need.

Chapter 2 provides an outline of this dissertation, which is subdivided in three parts: locoregional treatment developments in advanced melanoma, BRAF treatment in advanced melanoma and developments in the treatment of patients with melanoma brain metastasis.

Chapter 3 answers the long standing question whether sentinel lymph node biopsy (SLNB) causes intralymphatic metastasis (ILM). Previous studies have found an increased number of ILM after performing a SLNB and it has been postulated that this procedure leads to entrapment of tumor cell emboli in dermal lymphatic vessels. A systematic review and meta-analysis were performed of data on 33,622 patients treated in 36 studies. Upon meta-analysis, the total number of ILM as first recurrence after any surgical procedure performed to remove the primary tumor was 3.4%. ILM most frequently occurred after wide local excision (WLE) and a positive SLNB (13.2%), followed by WLE/delayed lymph node dissection (5.5%), WLE/elective lymph node dissection (4.7%), WLE/negative SLNB (3.4%) and WLE alone (1.9%). Insufficient data were available on therapeutic lymph node dissections. Differences in ILM occurrence between WLE and WLE/SLNB groups were statistically significant, as were differences between a positive SLNB/completion node dissection and elective lymph node dissection, leading to the conclusion that a SLNB alone is associated with an increase in the risk of ILM. As intralymphatic metastases are more common after a positive SLNB, which is commonly followed by a completion lymph node dissection, than after elective lymph node dissection, the stasis hypothesis does not hold true; after all, elective lymph node dissection leads to similar disruption of lymph flow as a positive SLNB plus completion node dissection. The increased risk of ILM is therefore associated with aggressive tumor biology.

Regional therapies for metastatic melanoma are explored in **chapter 4**. For patients with metastases confined to the extremities or the liver, regional perfusion and infusion techniques may be a valid option for treatment, with less morbidity than systemic treatment. Intra-arterial regional therapy of the limb using vascular isolation and chemotherapy, most commonly melphalan, can be done using two modalities: (1) (hyperthermic) isolated limb perfusion, creating venous and arterial access through a surgical incision in lower abdomen, groin, distal thigh, axilla, or upper limb for iliac-, femoral, popliteal, axillary or brachial perfusion, or (2) isolated limb infusion of the upper- or lower extremity, using an endovascular technique. Durable complete response rates of 40-80% and 30-38% have been reported, respectively. Tolerated doses of melphalan are 10- to a 100-fold higher in regional than in systemic administration. For isolated liver metastasis, isolated liver perfusion, using a laparotomy and requiring a prolonged hospital stay, and percutaneous liver perfusion have been used. Liver metastases obtain inflow primarily from the hepatic artery, so that chemotherapy can be directly infused into the hepatic artery and directed at the tumor. Vascular isolation can be achieved via balloon occlusion of the inferior vena cava, also allowing for filtration of the chemotherapy with a veno-venous bypass. Percutaneous hepatic perfusion has shown response rates of 34% for uveal and cutaneous melanoma in a randomized phase 3 trial, as opposed to 2% for best alternative care (n = 93). Isolated limb infusion/perfusion and percutaneous hepatic perfusion can be repeated multiple times in the same patient. In isolated melanoma of limb or liver, regional therapy therefore is an important option to consider, given the option of a closed circuit treatment and possibly postponing of systemic treatments with more toxicities. This applies in particular to the minimally invasive techniques.

Chapter 5 encompasses a state of the art overview of intralesional therapy for intralymphatic metastases. Control of intralymphatic disease potentially offers a benefit for selected patients because a subgroup will not develop distant metastasis. Patients with limited locoregional disease often experience relatively few symptoms, which may make regional infusion and perfusion procedures and systemic therapies that could expose patients to less adverse events. A number of agents have been investigated for intralesional administration; most data are available for velimogene aliplasmid, Bacille-Calmette-Guerin (BCG), electrochemotherapy with bleomycin or cisplatin, granulocyte macrophage colony-stimulating factor (GM-CSF), IL-2, PV-10 (a 10% solution of rose bengal) and talimogene laherparepvec (TVEC). These agents have shown response rates up to 99% in phase 1/2 trials of varying quality and case series. Velimogene aliplasmid, PV-10 and TVEC have shown a bystander effect where uninjected distant lesions have shown responses, which is particularly desirable in intralesional treatment. Several agents have been abandoned: BCG due to its adverse event profile, IL-2 due to its high cost and labor-intensive administration scheme. Electrochemotherapy has shown promising results, but data so far are insufficient to apply this approach broadly in clinical practice. Velimogene aliplasmid, GM-CSF and TVEC have been studied in randomized phase 3 trials. Velimogene aliplasmid failed to show an improvement in overall response rate (ORR) or overall survival (OS). For PV-10, a phase 3 trial is ongoing. GM-CSF was proven inferior to TVEC in a phase 3 trial, where TVEC showed an 36.1% ORR (vs. 0% with GM-CSF), with 36.1% of patients experiencing durable responses (i.e. >6 months). This shows that intralesional therapy is a valuable asset to the treatment palette for advanced melanoma, with PV-10 and TVEC showing the most promising results for clinical application.

BRAF-targeted therapy could be of benefit as a neoadjuvant strategy in selected unresectable patients, as explored in **chapter 6**. Currently, there are no approved neoadjuvant therapies for melanoma. We identified 15 patients with unresectable locoregional disease who were

treated with BRAF-targeted therapy with the intention of rendering them resectable in a single institution. Out of these, 11 patients used vemurafenib, three patients received dabrafenib/trametinib and one patient participated in a trial where patients were randomized between dabrafenib plus trametinib or dabrafenib plus placebo. After a median follow-up of 25.4 months after initiation of BRAF-targeted therapy, six patients proceeded to surgical resection after a median of 4.7 months of treatment. Histopathologic evaluation of resected disease showed complete response in two patients, partial response in two patients and no response in two patients. These responses only partially correlated to RECIST-based radiological evaluation. Four out of the six resected patients have been alive for more than two years since start of therapy, two out of which have not relapsed after 24.9 and 39.5 months, both after receiving dabrafenib/trametinib. This demonstrates that neoadjuvant BRAF-targeted therapy is a viable treatment strategy in select patients and that the decision to proceed to surgery should not solely depend on radiological evaluation.

Chapter 7 gives an overview of long-term effects of BRAF inhibitors in melanoma. Although the majority of patients experiences progression after a median of 7-9 months (BRAF monotherapy) and nine months (combined BRAF and MEK therapy), an increasing number of long term survivors are reported. However, BRAF-targeted therapy has been observed to lead to paradoxical MAPK pathway activation in BRAF wild-type and low-activity mutant cells, which is thought to be involved in the paradoxical oncogenesis seen during vemurafenib and dabrafenib treatment. This paradoxical MAPK activation may lead to secondary malignancies. Proliferative toxicities, such as squamous cell carcinoma, keratoacanthoma and verrucal keratosis are seen very frequently. Due to the brief time of onset (days to weeks), BRAF-targeted therapy is thought to potentiate preexisting initiating oncogenic events, more so than having direct oncogenic effects. BRAF treatment seems to be able to provoke previous existing or dormant RAS mutant cancers, as other interesting clinical findings have included colonic and gastric polyps, recurrent KRAS mutant colon cancer and RAS mutant leukemia. Combining BRAF inhibitors with MEK inhibitors, thereby inhibiting the MAPK pathway on multiple levels, leads to a decrease in paradoxical oncogenic development (the incidence of squamous cell carcinomas decreases from 19% for dabrafenib alone to 2-7% for dabrafenib/trametinib), however, MEK inhibitors come with side effects of their own.

Finally, **chapter 8** deals with one of the most clinically challenging scenarios that physicians face: patients with melanoma which has disseminated to the brain. In an extensive database consisting of 610 melanoma patients who initiated systemic treatment between 2000 and 2012, of which 243 were diagnosed with melanoma brain metastasis (MBM), data were collected on patient characteristics, staging, systemic therapies, MBM status/management, and survival. The risk of developing *de novo* MBM was similar in patients with chemotherapy, biochemotherapy, BRAF-targeted therapy, ipilimumab and anti-PD-1/PD-L1 regimens. MBM patient median OS was significantly shorter when determined from time of first regional/distant metastasis but not from time of first systemic therapy. Median OS from time of MBM diagnosis was 7.2 months, 8.4 months and 22.8 months for patients diagnosed 2000-2008, 2009-2010, and 2011-present, respectively (p = 0.002), which led to the following key observations: (1) the overall incidence of *de novo* MBM in patients with advanced melanoma receiving systemic therapy was 40%, which primarily occurred prior to or during the first line of therapy; (2) the incidence of MBM was not significantly different with BRAF-targeted agents, ipilimumab or anti-PD-1/PD-L1 therapy compared to traditional chemotherapy; (3) the median OS of MBM patients was statistically shorter than MBM-free patients from time of first regional or distant metastasis but not from start of first-line systemic therapy; and (4) the median OS of MBM patients was significantly longer in patients diagnosed with MBM in 2011 or after, which was independent of other MBM prognostic factors.

Nederlandse samenvatting (Dutch summary)

Het melanoom en paradigmata in de huidige behandeling worden uitgebreid geïntroduceerd in **hoofdstuk 1**, met een focus op het cutaan melanoom, het meest voorkomende subtype van deze tumor. De introductie bestaat uit een korte geschiedenis van het melanoom, een overzicht van epidemiologie, prognose, klinische presentatie, stadiëring, pathologie en moleculaire biologie en een overzicht van de behandeling: chirurgisch, regionaal, intralesionaal en systemisch. Een separate sectie gaat in op een van de grootste klinische uitdagingen heden ten dage, namelijk hersenmetastasen van het melanoom.

Hoofdstuk 2 geeft een overzicht van dit proefschrift, dat is onderverdeeld in drie delen: ontwikkelingen in de locoregionale behandeling van het gemetastaseerd melanoom, behandeling met BRAF-remmers en ontwikkelingen in de behandeling van patiënten met hersenmetastasen.

Hoofdstuk 3 beantwoordt de vraag of de schildwachtklierprocedure leidt tot intralymfatische metastasen (intralymphatic metastases (ILM)). Eerdere studies hebben een verhoogd percentage ILM gevonden na het uitvoeren van een schildwachtklierbiopsie en het is gepostuleerd dat deze procedure leidt tot het insluiten van tumorcelembolieën in de dermale lymfevaten middels disruptie van de lymfestroom (stase hypothese). Een meta-analyse en systematische review werden uitgevoerd op data van 33.622 patiënten, behandeld in 36 studies. De meta-analyse toonde een incidentie van 3,4% voor ILM als eerste recidief na een chirurgische ingreep ter behandeling van de primaire tumor. ILM deden zich het vaakst voor na lokale excisie in combinatie met een positieve schildwachtklierbiopsie (13,2%), gevolgd door lokale excisie in combinatie met een vertraagde lymfeklierdissectie (5,5%), lokale excisie in combinatie met een electieve lymfklierdissectie (4,7%), lokale excisie in combinatie met een negatieve schildwachtklierbiopsie (3,4%) en lokale excisie alleen (1,9%). Voor de therapeutische lymfeklierdissectie waren onvoldoende gegevens beschikbaar. Het verschil in ILM-incidentie tussen lokale excisie en lokale excisie in combinatie met zowel positieve als negatieve schildwachtklierprocedure waren statistisch significant, hetgeen leidt tot de conclusie dat een schildwachtklierbiopsie inderdaad geassocieerd is met een ILM verhoogd risico. Echter, het feit dat de incidentie van ILM hoger is na een positieve schildwachtklierprocedure (vaak gevolgd door aanvullende lymfeklierdissectie), dan na electieve lymfeklierdissectie, levert bewijs tegen de stase hypothese. Electieve lymfeklierdissectie leidt tot gelijksoortige disruptie in de lymfestroom als een positieve schildwachtklierprocedure aangezien bij een positieve schildwachtklier een aanvullende lymfeklierdissectie wordt uitgevoerd. De meest voor de hand liggende etiologie is dan ook dat het verhoogde ILM-risico is geassocieerd met agressieve eigenschappen van de primaire tumor en niet met de schildwachtklierprocedure.

Regionale therapieën als behandeling voor uitgezaaid melanoom worden samengevat in **hoofdstuk 4**. Voor patiënten met metastasen beperkt tot de ledematen of de lever kunnen regionale perfusie- en infusietechnieken een goede behandeloptie bieden, met minder morbiditeit dan systemische behandeling. Er zijn twee modaliteiten beschikbaar voor intra-arteriële regionale behandeling van de ledematen met chemotherapie (meestal melfalan): (1) (hyperthermische) geïsoleerde ledemaatperfusie, waarbij een veneuze en arteriële toegang wordt gecreëerd via een chirurgische incisie in de onderbuik, lies, oksel, of bovenste ledemaat of (2) geïsoleerde ledemaatinfusie van de extremiteiten met behulp van een endovasculaire techniek. Bij beide technieken wordt gebruik gemaakt van vasculaire isolatie. Duurzame volledige responsen van 40-80% en 30-38% zijn gemeld voor perfusie en infusie. Het voordeel van regionale behandeling met melfalan is dat het mogelijk is een 10- tot 100-voudige dosis te gebruiken ten opzichte van wat getolereerd wordt bij systemische toediening. Voor geïsoleerde levermetastasen zijn klassieke leverperfusie en percutane leverperfusie beschreven. De laparotomie die noodzakelijk is voor een klassieke leverperfusie leidt tot een langer verblijf in het ziekenhuis en meer morbiditeit in vergelijking met de percutane procedure. Levermetastasen verkrijgen hun bloed voornamelijk via de arteria hepatica, zodat chemotherapie lokaal in de arteria kan worden toegediend en hoge doses in de tumor worden bereikt. Vasculaire isolatie kan worden bereikt door ballonocclusie van de vena cava inferior, hetgeen ook de filtratie van chemotherapie met een veno-veneuze bypass mogelijk maakt. Percutane leverperfusie heeft een aangetoond responspercentage van 34% voor uveaal en cutaan melanoom in een gerandomiseerde fase 3 studie, tegenover 2% voor de beste alternatieve zorg (n = 93). Zowel geïsoleerde ledemaatinfusie, percutane leverperfusie en geïsoleerde ledemaatperfusie kunnen meerdere keren worden herhaald in dezelfde patiënt. Wanneer metastasen zijn beperkt tot een extremiteit of de lever is regionale therapie dan ook een belangrijke optie ter overweging, met uitstel van systemische behandelingen met meer toxiciteit. Dit geldt met name voor de minimaal invasieve technieken.

Hoofdstuk 5 bevat een overzicht van intralesionale therapie voor in-transit metastasen. Een beperkte groep patiënten ontwikkelt recidiverende in-transit metastasen zonder metastasen op afstand. Patiënten met beperkte locoregionale ziekte hebben veelal relatief weinig symptomen. Dat maakt de toxiciteit die gepaard gaat met systemische therapie en zelfs met regionale infusie- en perfusietechnieken minder aantrekkelijk. Een aantal medicijnen is onderzocht voor intralesionale toediening; de meeste gegevens zijn beschikbaar voor velimogene aliplasmid, Bacille-Calmette-Guerin (BCG), elektrochemotherapie met bleomycine of cisplatine, granulocyt macrofaag-kolonie stimulerende factor (GM-CSF), IL-2, PV-10 (een 10% oplossing van rose bengal) en talimogene laherparepvec (TVEC). Deze medicijnen hebben aangetoonde responspercentages tot 99% in fase 1/2 studies van wisselende kwaliteit en case-series. Velimogene aliplasmid, PV-10 en talimogene laherparepvec hebben bovendien een bijstandereffect, waarbij niet geïnjecteerde laesies op afstand een respons vertonen. Dit bijstandereffect is een zeer wenselijke eigenschap van intralesionale therapie. Voor verscheidene middelen is de verdere ontwikkeling gestaakt:

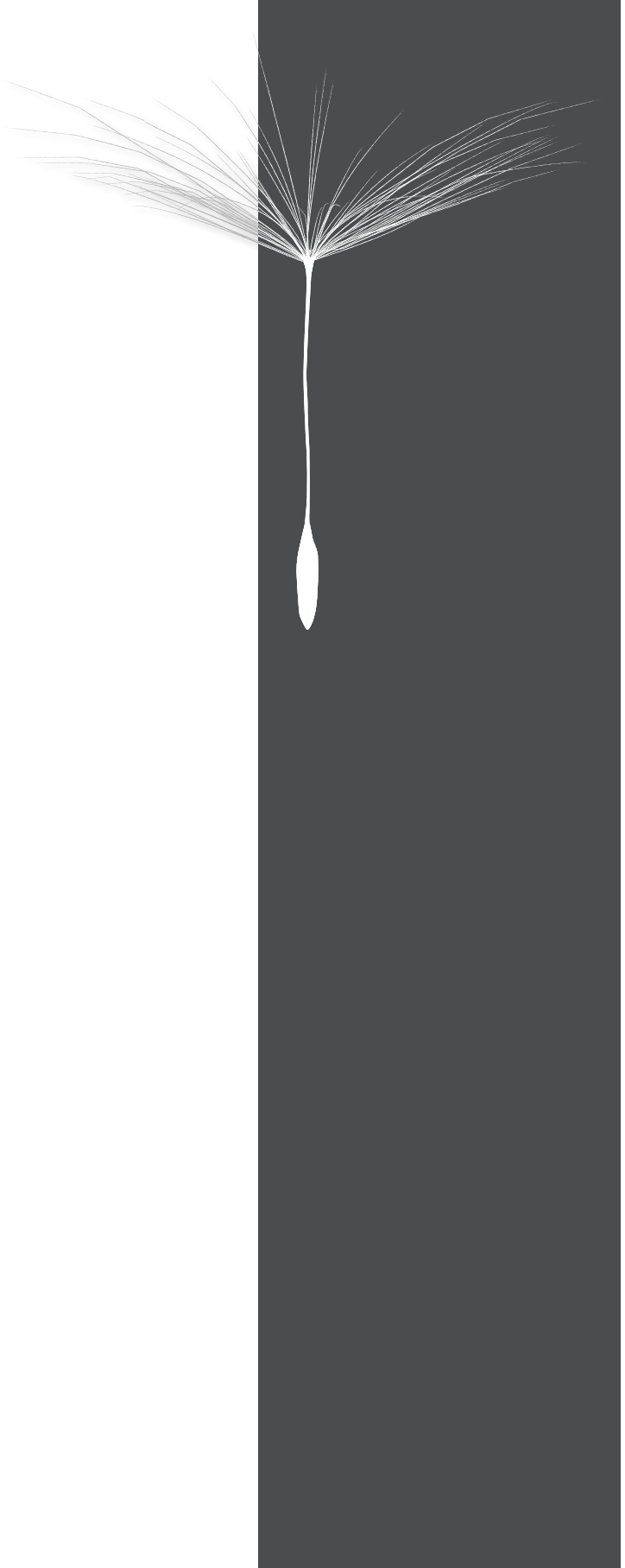
BCG vanwege zijn bijwerkingenprofiel, IL-2 vanwege de hoge kosten en arbeidsintensief toedieningsschema. Elektrochemotherapie laat veelbelovende resultaten zien, maar er zijn onvoldoende studies beschikbaar om deze methode breed in te zetten in de klinische praktijk. Velimogene aliplasmid, GM-CSF en talimogene laherparepvec zijn getest in gerandomiseerde fase 3 studies. Velimogene aliplasmid leidde niet tot verbeterde Overall Response Rate (ORR) of Overall Survival (OS). PV-10 bevindt zich nog in fase 3. GM-CSF is bewezen inferieur aan TVEC; de gerapporteerde ORR is 6% vs. 26%. Van de TVEC-patiënten ervoer 16% een duurzame reactie (d.w.z. respons ≥6 maanden). Dit toont aan dat intralesionale therapie is een waardevolle aanwinst is in de behandeling van gevorderd melanoom, met PV-10 en TVEC als veelbelovende medicatie voor klinische toepassing.

BRAF- en MEK-remmers kunnen potentieel ingezet worden als neoadjuvante therapie voor patiënten met inoperabel melanoom. Dit wordt geëxploreerd in **hoofdstuk 6**. Er zijn momenteel zijn geen goedgekeurde neoadjuvante therapieën voor melanoom. We identificeerden 15 patiënten met inoperabele locoregionale ziekte die behandeld waren met BRAF-remmers met of zonder MEK-remmer met als doel de tumor alsnog in zijn geheel te reserceren. Van deze 15 patiënten gebruikten er 11 vemurafenib, drie patiënten kregen dabrafenib/trametinib en één patiënt nam deel aan een studie met randomisatie tussen dabrafenib/trametinib of dabrafenib/placebo. Na een mediane follow-up van 25,4 maanden na start van de systemische behandeling kon bij zes patiënten alsnog worden overgegaan tot chirurgische resectie, na een mediane behandelingsduur van 4,7 maanden. Histologische evaluatie van gereserceerde ziekte vertoonde complete respons in twee patiënten, partiële respons in twee patiënten en geen respons in twee patiënten. Deze bevindingen kwamen slechts gedeeltelijk overeen met op RECIST gebaseerde radiologische evaluatie. Twee jaar na start van de systemische behandeling waren vier van de zes geopereerde patiënten nog in leven; waarvan twee zonder recidief na 24,9 en 39,5 maanden follow-up. Beide patiënten werden behandeld met dabrafenib/trametinib. Dit toont aan dat BRAF- en MEK-remmers een optie zouden kunnen zijn voor een neoadjuvante strategie, maar ook dat de beslissing om over te gaan tot een operatie niet gebaseerd moet zijn op radiologische evaluatie alleen.

Hoofdstuk 7 geeft een overzicht van de lange-termijneffecten van BRAF-remmers bij behandeling van het melanoom. Hoewel de mediane progressievrije overleving respectievelijk 7-9 maanden (BRAF monotherapie) en negen maanden (combinatietherapie met BRAF- en MEK-remmer) bedraagt, zijn patiënten beschreven die langdurig in remissie blijven op therapie. Behandeling met BRAF-remmers kan echter leiden tot paradoxale activatie van de MAPK-pathway in cellen met wild-type-BRAF en BRAF-gemuteerde cellen met lage activiteit. Dit mechanisme speelt vermoedelijk een rol in de paradoxale oncogenese die wordt gezien tijdens behandeling met vemurafenib en dabrafenib. Deze paradoxale MAPK-activering kan leiden tot secundaire maligniteiten. Proliferatieve toxiciteiten, zoals plaveiselcelcarcinoom, keratoacanthoom en verruceuze keratosis, zijn een frequente bijwerking. Aangezien deze oncogenese relatief snel (dagen-weken) optreedt na start van BRAF-remmende therapie ligt het voor de hand dat BRAF-remmers niet zozeer directe oncogene effecten hebben, maar preëxistente oncogene events in de cel versterken. Andere interessante klinische bevindingen zijn de ontwikkeling van colon- en maagpoliepen, een recidief van KRAS-gemuteerd coloncarcinoom en optreden van RAS-gemuteerde leukemie. Het combineren van BRAF-remmers met MEK-remmers, en daarmee het remmen van de MAPK-pathway op meerdere niveaus, leidt tot een afname in deze paradoxale oncogene ontwikkeling. Zo daalt de incidentie van plaveiselcelcarcinomen van 19% voor dabrafenib alleen tot 2-7% voor dabrafenib/trametinib. MEK-remmers hebben echter hun eigen bijwerkingen.

Tot slot gaat **hoofdstuk 8** in op één van de grootste klinische problemen waar artsen mee worden geconfronteerd in de behandeling van het melanoom: patiënten die hersenmetastasen ontwikkelen. We includeerden 610 melanoompatiënten die hun systemische behandeling startten tussen 2000 en 2012 in een database. Van deze groep werden er 243 gediagnosticeerd met hersenmetastasen (melanoma brain metastasis, MBM). Gegevens werden verzameld met betrekking tot de patiëntkenmerken, stadiëring, systemische behandeling, MBM-status en behandeling, en overleving. Het risico van *de novo* MBM was vergelijkbaar onder patiënten die behandeld werden met chemotherapie, biochemotherapy, BRAF-remmers, ipilimumab en anti-PD-1-/PD-L1-regimes. De mediane OS van MBM-patiënten was significant korter dan die van de controlegroep, wanneer gemeten vanaf het tijdstip dat de eerste metastase op afstand zich ontwikkelde, maar niet vanaf het tijdstip waarop begonnen was met systemische therapie. De mediane OS vanaf het tijdstip van MBM-diagnose was 7,2 maanden; 8,4 maanden en 22,8 maanden voor patiënten gediagnosticeerd van 2000-2008, 2009-2010 en 2011-heden (p = 0,002). Analyses uit de database leidden tot de volgende conclusies: (1) de incidentie van *de novo* MBM bij patiënten met gevorderd melanoom was 40%, waarbij MBM voornamelijk optrad voor aanvang van systemische behandeling of tijdens de eerste lijn; (2) de MBM-incidentie was niet significant verschillend tussen patiënten behandeld met BRAF-remmers, ipilimumab of anti-PD-1/PD-L1 therapie in vergelijking met chemotherapie; (3) de mediane OS van patiënten zonder MBM was langer dan die van MBM-patiënten wanneer gerekend vanaf het tijdstip van de eerste uitzaaiing, regionaal danwel op afstand, maar niet wanneer gerekend vanaf initiatie van systemische therapie; en (4) de mediane OS van MBM patiënten was significant hoger in patiënten die gediagnosticeerd werden met MBM in of na 2011, onafhankelijk van andere prognostische factoren.

10 Discussion and future perspectives



Discussion and future perspectives

Introduction

The field of melanoma treatment has never before seen such a relative abundance of treatment strategies as today. This holds true for surgical management, locoregional treatments and systemic treatments alike. The rapid developments described in this dissertation have opened up new options for patients with intralymphatic metastasis, (neo)adjuvant treatment for (bulky) regional disease and (combined) treatment options with palliative or curative intent for disseminated disease.

In the upcoming decade on the surgical side we will see further refinement of minimally invasive techniques, intralesional therapies, and infusion and perfusion approaches, establishing whether there are patient groups for which less extensive curative and palliative procedures with less morbidity suffice. From the medical approach, advancement will come from sequencing therapies, identification of predictive biomarkers and combination treatment approaches. Our understanding of the disease will increase as we learn more about the interaction of aberrant and adaptive molecular pathways along with immune phenotypes; this will be balanced by the discussions on economic feasibility and utilizing positive and negative predictive biomarkers in making treatment decisions. Together these developments will lead to an approach that is more individualized than the current paradigms.

In surgery, less is more

In surgery, we are moving towards a more patient-centric, 'less is more' approach. After the proven benefit of wide excision margins and sentinel lymph node biopsy (SLNB) with completion node dissection, trials are now focusing on selecting for which patients excision margins can safely be reduced to 1 mm and which patients do not need a completion node dissection after SLNB.[1-4] Moreover, the development of robotic and videoscopic surgical techniques allow for less invasive approaches.[5,6] Time and trials will tell which patients and procedures benefit from these techniques.

In locoregional treatment: moving towards evidence for PFS/OS benefit

When metastatic melanoma is limited to the limb or liver, regional therapy is an important option to consider, especially the hyperthermic isolated limb perfusion (HILP), minimally invasive isolated limb infusion (ILI) and percutaneous hepatic perfusion (PHP) techniques.[7-10] The benefits these procedures provide include a percutaneous approach that avoids the morbidity of open and complex surgical procedures, the ability to perform multiple treatments in the same patient and the ability to avoid or postpone systemic treatments

which have more toxicity. HILP, ILI and PHP have demonstrated efficacy in achieving regional control of disease. Because of the readily accessible bypass circuit, real time pharmacokinetic data can be easily obtained. HILP and ILI also have the added feature of providing access to tumor tissue in the treated field during the entire time course of treatment via tumor biopsy of subcutaneous lesions with minimal morbidity. As more data become available, we will learn whether PHP provides PFS and OS benefits in addition to improved response rates.
Intralesional therapy is an attractive option for patients with in-transit disease when surgical resection to render a patient free of disease is not feasible. The ideal agent should have a low toxicity profile, be easy to administer, lead to fast responses and trigger a systemic immune response, thereby creating a bystander effect. Intralesional injections are particularly attractive due to the fact that they are generally very well tolerated, they can be done during an outpatient procedure, and their ability to produce durable responses, albeit in a modest percentage of patients. The aforementioned bystander effect is mainly seen with PV-10 (rose bengal) and TVEC and makes these agents particularly attractive. TVEC is the first therapy to have shown an OS benefit in patients with stage IIIB/IIIC and M1A disease.[11]

In systemic treatment: sequencing, biomarkers and quality of life

While the initial development of targeted therapy strategies was limited by a relative absence of therapeutic agents, the current challenge for both surgical and medical oncologists is to prioritize agents for systemic treatment when there are multiple physiological and pathological mechanisms to target. As there are growing examples of the critical nature of the degree of target inhibition, differences in pharmacokinetic properties and/or drug delivery methods are key issues, particularly in the development of systemic therapies for brain metastases. The achievement of durable clinical benefit requires an understanding of the mechanisms that underlie resistance in order to develop rational and effective strategies to prevent and/or overcome them. Recent melanoma exome sequencing efforts have failed to identify new frequently mutated kinase targets. New insights into the prognosis of people with metastatic melanoma might come from molecular profiling of the primary tumor and distant metastases, identifying the range of mutations along with the immunophenotype.

The availability of patient subsets surviving long term is another factor that will increase our understanding of melanoma biology. Before the introduction of the new wave of systemic treatments, less than 12% of patients were alive beyond five years and prospects for patients with brain metastases were even bleaker still. It is now seven years ago that the first BRAF inhibitor, vemurafenib, and the anti-CTLA-4 antibody ipilimumab were approved.[12] Survival of patients treated with ipilimumab seems to plateau after three years at around 20%.[13] For vemurafenib, long term survival has been observed as well, with 3- and 4-year melanoma specific survivals of 26% and 19% being reported.[14] Anti-PD1 therapy offers even better prospects, with 35% of the phase 1 trial patients surviving after five years and a plateau being reached after four years.[15] Research is focusing on combination therapies to further improve these outcomes and overcoming resistance. One area of research focuses on enhancing the effect of anti-CTLA-4 and anti-PD1/PD-L1 agents by adding immune stimulating antibodies such as TVEC or IDO inhibitors.[16,17] However, improved outcomes may come at the price of higher toxicity, as seen with the ipilumumab/nivolumab combination.[18,19] Quality of life must not be ignored and will factor into treatment decisions.

Increasingly: individualized cancer care

Individualized cancer treatment is an important cornerstone in the current treatment landscape. BRAF-targeted treatment is a beautiful example, where about 50% of patients harbor the BRAF V600E mutation.[20] However, for anti-PD-1 therapy, this is not as clear cut. Although patient's populations with high PD-L1 tumor expression typically have higher responses rates and survival with anti-PD-1 therapies, patients with low PD-L1 tumor expression can still benefit from anti-PD-1 therapy.[21,22] Better predictive biomarkers are needed for immunotherapy approaches. In addition, molecular alterations in the P13K pathway have not proven to be successful therapeutic targets in patients with advanced melanoma, indicating further work is needed. A better understanding of the pathways involved in melanogenesis and the increasing availability of next generation sequencing and other assays will lead to truly personalized medicine. Studies like NCI-MATCH (NCT02465060) and TAPUR (NCT02693535) signal a new era in precision medicine, where therapies are tailored to specific mutations as opposed to disease state.

In radiotherapy: highly focused technologies

Despite the historical concept of melanoma as a radiotherapy resistant tumor, new paradigms that employ radiation therapy (RT) to treat melanoma are rapidly emerging. The increasing understanding of the role of the immune system in regulating the response to RT and the recent development of a multitude of immuno-oncologic treatment modalities might change the role of RT in melanoma treatment. Highly focused RT including intensity modulated radiation therapy (IMRT), 3D conformal radiation therapy (3DCRT), stereotactic radiation therapy (SRT) and proton therapy, which will become available at the University of Groningen at the end of 2017, allow for dramatic dose escalation due to the ability of these techniques to improve the precision with which radiation therapy can be administered and avoid dose-limiting tissue structures.[23-26]

And last but not least: cost of cancer care and the continued importance of primary prevention

Despite the large improvements that have been made in the medical and surgical management of patients with advanced melanoma, we must not forget that the majority of stage IV patients still succumb to their disease. Median overall survival rates for the nivolumab/ipilimumab combination are eagerly awaited, as are prospective overall survival data for anti-PD1 treated patients with brain metastases.[18,19,27] An abundance of immune modulatory agents are now seen in pharmaceutical pipelines, which raises questions on how to optimize combinations,

sequencing and cost of cancer care. The financial burden of melanoma (and other cancer) treatments will likely rise.

Primary prevention is still an important part of the melanoma landscape and should not be overlooked. It is well known that smoking increases the risk for lung cancer and a recent study showed that smoking is also associated with an increased risk of experiencing lymph node metastasis in patients with melanoma.[28] Furthermore, the importance of sun-protective behavior should continue to be stressed, especially for children and adolescents.[29]

Taking all of these factors into account, treatment of melanoma has become increasingly complex and will continue to do so. Treatment requires an individualized and multidisciplinary approach. The ideal treatment should be tailored to the individual patient and based on the extent of disease, tumor characteristics, such as BRAF status and disease free interval, and patient characteristics including age and comorbidities. This will lead to a combination of injectable treatments, regional perfusions/infusions and systemic treatment. Imhotep's treatment statement, "There is none", finally stands defeated.

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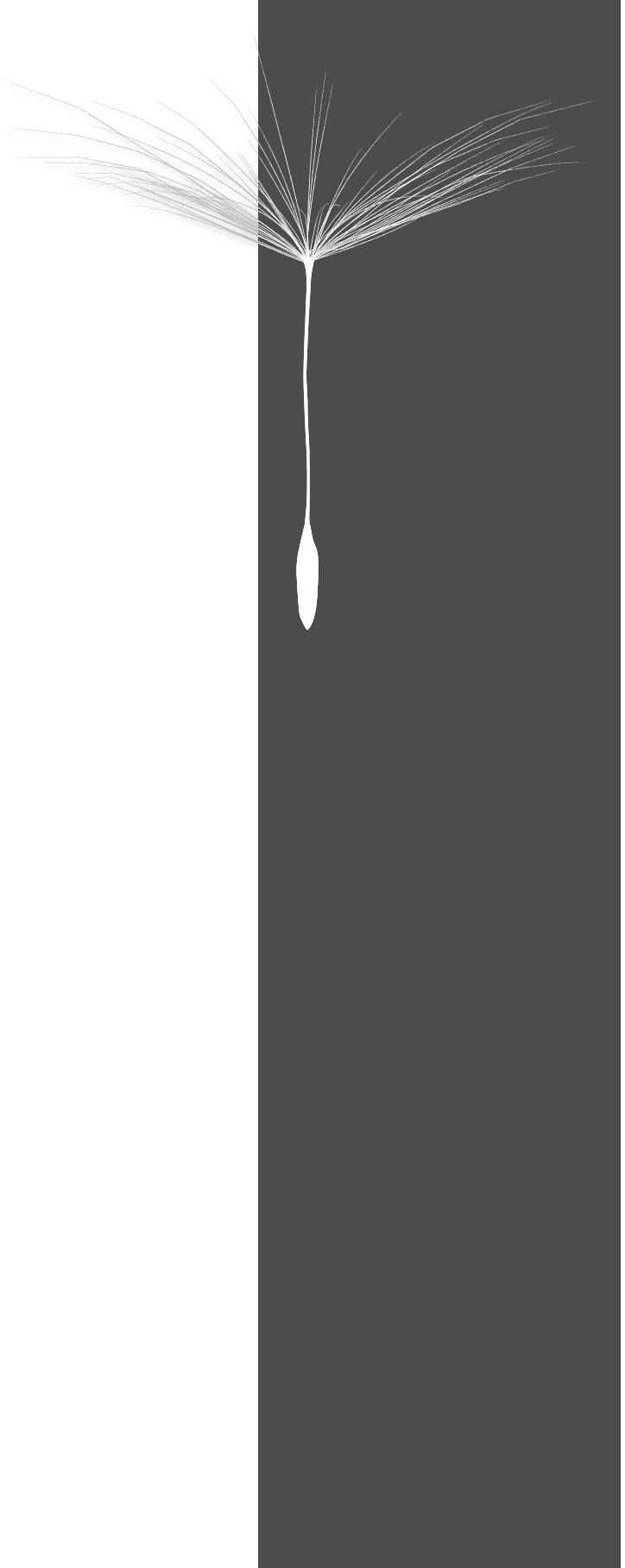
11 Appendices



Curriculum Vitae

Sarah Sloot was born on April 14th 1988 in Walenstadt, Switzerland. She is the eldest daughter of Gertie Botter and Luigi Tosolini and has six siblings: Laura, Larissa, Emma, Julia, Jesse and Fabian. After completing high school (Gymnasium, CSG Het Noordik, Almelo), she studied Medicine at the Radboud University in Nijmegen. She participated in the Radboud Honors Program.

As a self-proclaimed workaholic, throughout her studies Sarah was actively involved in a number of committees; amongst others, she organized the LOCA symposium, the largest national symposium for medical students, she was the treasurer for Dance Fever, the second largest students' sports union in Nijmegen, and she served as chairman of the student magazine 'Status Co', specifically for medical students. Next to that, she worked in home care assisting the elderly, gave tours at the Radboud Museum of Anatomy and Pathology and worked at the venipuncture laboratory of the Radboud University Hospital. She completed her studies with an internship at Medisch Contact, a magazine weekly distributed by the Dutch Royal Society for Improvement of Medicine, before graduating in 2011.

Faithful to her curious and investigative nature, Sarah started doing research during her master's degree and decided to pursue a PhD under the guidance of Prof. dr. H.J. Hoekstra, after she started working as an intern ('AGNIO)' in the Isala Klinieken in Zwolle, where Dr. E.G.J.M. Pierik was her supervisor. With the support of the Groningen Melanoma and Sarcoma Foundation she spent a year at Moffitt Cancer Center in Tampa, Florida, USA, as an exchange PhD student from the University of Groningen. Upon returning to The Netherlands, Sarah started working as an intern at the Department of Surgery at the University Medical Center in Groningen, after which she pursued a job as a Medical Advisor in Oncology at Pfizer. In 2016, she decided to move to the United States to be with her husband, whom she had met during her exchange year. She continues to happily work for Pfizer as a Field Medical Director in the field of oncology, where her heart lies, and aims to combine living in sunny Florida with an excellent job performance and traveling the world.

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First of all I would like to thank my promotor, Prof. dr. Harald J. Hoekstra, for mentoring and enabling me with the chance of going abroad to broaden my horizon and gain new insights. The year I spent in the USA gave me so much more than I bargained for! I got the opportunity to work at a highly specialized oncologic center together with very skilled colleagues and I expanded my knowledge of the molecular biology of melanoma. I developed a newfound respect for just how much effort it takes to develop new medication and how much brain- and manpower that medication subsequently needs to make the jump from the laboratory to the clinic, and eventually to the common practice.

Special thanks go to the Groningen Melanoma and Sarcoma Foundation, which granted me the funding to make it possible to study abroad and which continues to support talent to pursue research and add to the existing body of data. Thank you for providing students with new opportunities.

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Of course even after the research had been completed and all articles featured in this dissertation had been submitted, I was far from done. David Woods and Daniel Verduszco proofread the original manuscript and helped with irregularities I could no longer see. Tough love is not always appreciated in the moment itself, however, as time goes by its true value shows. Harald Hoekstra, Jonathan Zager and Geoffrey Gibney provided advice on the finishing touches. After that, Fleur Bominaar did a fantastic job on the design and lay-out of this finished volume before you. Thank you for helping to turn a paper pile full of words into a complete manuscript worth reading.

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My family has not only supported me throughout my dissertation, but more than that: throughout my life, which has been a rollercoaster more often than not. To my closest family: my mother, Gertie Botter, my sisters, Laura, Emma and Julia, and my brother, Jesse: thank you. Please visit often. I would like to mention my aunt Margreet Botter in particular, who has taken it upon her shoulders to introduce me to new cuisines during life. Food is an important part of ones upbringing.

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