CONGRESSIONALLY DIRECTED MEDICAL RESEARCH PROGRAMS

PEER REVIEWED MELANOMA RESEARCH PROGRAM LANDSCAPE: RESEARCH AND FUNDING

2021

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SUMMARY:

The vision of the Peer Reviewed Melanoma Research Program (MRP) is to prevent melanoma initiation and progression. In 2020 there were an estimated 100,350 new cases of melanoma; a 9% increase in new cases since 2018. Melanoma diagnoses are increasing among active duty Service, with the greatest incidence rates in the Air Force, Navy, and the Marines. Active duty Service members may spend prolonged periods outside, especially during deployment to areas of high ultraviolet radiation. To address the need for earlier melanoma intervention, the MRP commits to enhancing United States (US) military personnel mission readiness and to diminishing the disease burden of melanoma on Service members, Veterans, and the American public. This Summary briefly describes knowledge, clinical, and patient outcome gaps within the field of melanoma. Each year the MRP will evaluate the gaps and strategically address those gaps that may advance the field toward eradicating melanoma. Investigators interested in funding opportunities should review the current program announcements to identify the specific focus areas to be addressed per fiscal year.

GAPS:

- Identify methods to decrease risk of melanoma development beyond sunscreen and protective clothing.
- Identify risk factor determinants for melanoma variants (e.g., uveal, acral, mucosal melanoma).
- Identify how the tumor microenvironment (e.g., stromal, immune, microbiome) impact tumor initiation, response to therapy, progression, and dormancy.
- Understand how precursor lesions and endogenous host factors may lead to melanomagenesis.
- Develop new decision making tools for the detection and diagnosis of melanoma that includes easily accessible technology (beyond the dermoscope) for primary care physicians and dermatologists.
- Develop prediction and surveillance tools for distinguishing patients at risk for recurrence and/or metastasis. Identify biological determinants to differentiate patient populations.
- Understand mechanisms that underlie metastatic spread to different (regional/nodal) sites or the different distant sites of metastasis from acral, mucosal, and uveal melanomas.
- Delineate the molecular pathways in the tumor microenvironment, immune response that influence metastatic spread, recurrence, and/or dormancy.

SECTION I: Melanomagenesis

The most aggressive form of skin cancer, melanoma, affects approximately 22 people out of 100,000 in the United States annually. Melanoma accounts for only 1% of all skin cancers diagnosed, but causes the majority of deaths.[1] Current operations across the globe by the Department of Defense (DOD) in areas of high ultra-violet (UV) radiation exposure may put at risk military Service members for melanoma development.[2] From 2005 through 2014, melanoma was the number one cancer diagnosis for active duty Service members. It is the fourth most diagnosed cancer in Veterans[3] and therefore presents a healthcare crisis for the DOD and the U.S. Department of Veterans Affairs. The term melanoma encompasses a number of diverse cancers, (Table I) all originating from melanocytes.

Table I: Types of Melanoma

Type	Area(s) of Body Affected	Incidence (% of All Melanomas)	Five-Year Survival Rate
EPITHELIUM ASSOCIATED			
Acral (Lentiginous)	Most common among darker skin types; found on the palms of the hands, soles of feet, and under the nails	2%-3%	80.3%
Amelanotic	Appears on the surface of the skin; lack of pigment (appears as a pink or red spot)	2%-8%	88%
Congenital Melanocytic Nevus (CMN)	Present at birth on the surface of the skin; large pigmented mole or birthmark	0.2%-6.0% of all newborns; 2%-3% of patients with large or giant congenital nevi develop melanomas Unknown[1]	Unknown
Desmoplastic	Found on the head and neck of elderly patients	<4% of primary cutaneous melanomas; 2.0 per million	67%-89%
Lentigo Maligna	Most often found in older adults on sun-exposed areas such as the arms, legs, face, ears, neck, and other areas of the upper torso	5%-15%	93.2%; if metastatic, between 9%-27%
Nodular	Appears on the surface of the skin, spreads to deeper layers; usually found on the torso, legs and arms, and scalp	10%-15%	64.6%
Spitzoid	Appears on the surface of the skin; resembles a benign skin mole (Spitz nevus); commonly found on the head, hands, or legs	Unknown[2]	Unknown[2]
Pediatric Spitzoid	Appears on the surface of the skin; resembles a benign skin mole (Spitz nevus); commonly found on the head, hands, or legs	Unknown[3]	88% in children (ages 0–10 years) with metastatic spitzoid melanoma; 49% in children aged 11-17 years

[1] Incidence varies enormously with the severity of the congenital phenotype.
[2] More common in children and young adults.
[3] Too little data available (rare).

Type	Area(s) of Body Affected	Incidence (% of All Melanomas)	Five-Year Survival Rate
Superficial Spreading	Outer layer of the skin	~70%	91.6%
NON-EPITHELIUM ASSOCIATED			
Blue nevus melanoma	Appears mainly on extremities, scalp, and buttocks, in mid- to deep-dermis, and resembles a blue-black-grey-black discolored nevus	Unknown[4]	Unknown[4]
Melanoma of internal organs	Mucosal surfaces of the body (head and neck nasal passages and oral cavity, vagina and vulva, anus, rectum, gastrointestinal tract, and other areas)	<2% Head and neck: (31%-55%) Vulvovaginal: (18%-40%) Anorectal: (17%-24%)	29.1%
Leptomeningeal Disease	Cancer cells migrate to the cerebrospinal fluid; metastases in brain, spinal cord, and/or in the leptomeninges	50%-60% of metastatic melanoma cases	Typically measured in weeks to a few months
Uveal and Conjunctival	Develops in the melanocytes the eye	3%-5%	Uveal: 83.5% Conjunctival: 75.7%

Melanocytes are found in the skin, eye, mucous membranes, meninges, hair follicles, and other tissues throughout the body. Within the basilar epidermis, melanocytes are a minority cell population and infrequently divide. Melanin is secreted by melanocytes in response to keratinocytes releasing α-melanocyte stimulating hormone (αMSH) due to DNA damage (i.e., UV-radiation induced).[4] MC1R (melanocortin 1 receptor) on melanocytes responds to the αMSH by activating the enzymes responsible for the synthesis of melanin, the production of which leads to increases of superoxide anions and hydrogen peroxide in melanocytes.[5] Melanin biosynthesis occurs within melanosomes to protect the cells from harmful oxidative damage.

There are two types of melanin: eumelanin, which is UV absorbent and pheomelanin, which is photo-unstable. Studies have shown that increased levels of pheomelanin in relation to eumelanin is a risk factor for the development of melanoma.[6,7] The pheomelanin-to-eumelanin ratio is critical to the protective function. Eumelanin characteristics include the ability to scatter and absorb UV radiation and scavenge reactive oxygen species (ROS), therefore offering protection to the keratinocytes and deeper layers of the skin.[5] The reason for the presence of pheomelanin is still under debate. Its biochemistry does not lend it to being a good protector against the effects of UV radiation. Melanocytes in persons with fairer skin and red hair respond with more pheomelanin secreted than eumelanin, hence the greater risk of skin cancers and melanoma. It is thought that pheomelanin may be beneficial for physiological processes that are UV-dependent, such as vitamin D production. Synthesis of pheomelanin causes an increase in ROS production and depletion of molecules that function to decrease oxidative damage such as glutathione.[5] Utilizing this knowledge and building on it may lead to better ways to prevent production and/or scavenge ROS to decrease the likelihood of UV damage and the development of melanoma (especially cutaneous melanomas dependent on chronic sun exposure).

[4] Too little data available. Only ~100 cases described in literature as of 2014.

Melanomagenesis, the process by which a normal melanocyte evolves into a cancer cell, is not a straightforward step-by-step process. Evidence points to the significance of ROS in melanomagenesis in chronically sun-exposed areas of the epithelium. As mentioned, melanin is produced in response to αMSH produced by keratinocytes due to DNA damage by UV light. Aberrant oxidative control and ROS may have significant outcomes to melanocytes and the potential for mutational events. Sources for ROS include UV radiation, environmental toxins and chemicals, ionizing radiation, and inflammation.[6]

Multiple genes and signaling pathways have been implicated in the genesis of melanoma. UV radiation is often cited by the popular media as the main cause of melanoma. The reality is far more complex and intricate. In fact, in some cases or types of melanoma, UV radiation is not suspected to be the instigator (e.g., anogenital melanomas or uveal melanomas). Multiple genes are vulnerable to mutation in melanocytes (Table II).

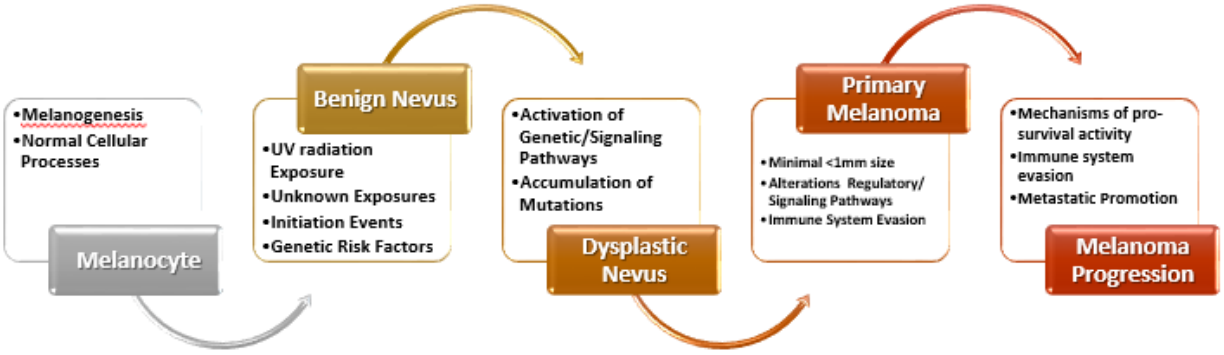
Table II: Genetics of Melanoma

Gene	Pathway	Functional Role	Sun Damaged	Melanomagenesis
BRAF	MAPK	Regulate proliferation, Signaling pathways involved in proliferation	Chronically exposed areas of skin; Non-chronically exposed areas of skin	Initiation; May be found in nevi, melanoma in situ, other intermediate lesions
NRAS	MAPK	Regulate proliferation, Metabolism, and Immune surveillance	Chronically exposed areas of skin	Initiation; May be found in nevi, melanoma in situ, other intermediate lesions
NF1	MAPK	Regulate proliferation, Immune surveillance	Chronically exposed areas of skin	Initiation; Found in melanoma in situ
TERT	Telomerase	Replicative lifespan	Chronically exposed areas of skin; Non-chronically exposed areas of skin	Initiation; May be found in nevi, melanoma in situ, other intermediate lesions
CDKN2A	RB	Cell cycle control	Chronically exposed areas of skin; Non-chronically exposed areas of skin	Progression; Found in invasive melanoma
ARID1/ ARID2	SWI/SNF Chromatin remodeling	Cellular identity	Chronically exposed areas of skin; Non-chronically exposed areas of skin	Progression; Transition to invasive melanoma
PTEN	PI3K	Growth and metabolism	Non-chronically exposed areas of skin	Advanced progression with thicker invasive melanomas
KIT	RAS/MEK	Growth and metabolism	Chronically exposed areas of skin; Non-chronically exposed areas of skin	Advanced progression; Found in acral, mucosal melanomas as well as sun-damaged skin
TP53	P53	Tumor suppressor	Chronically exposed areas of skin	Advanced progression with thicker invasive melanomas
INK4A	P16	Cell cycle control	Chronically exposed areas of skin; Non-chronically exposed areas of skin	Progression; Transition to invasive melanoma

Gene	Pathway	Functional Role	Sun Damaged	Melanomagenesis
GNAQ/ GNA11	Gαq	Regulation of development signaling pathways	Not related; uveal melanomas, blue nevi, and meningeal melanoma	Initiation in uveal melanoma, blue nevi, and meningeal melanoma
BAP1	BRCA	Tumor suppressor	Not related; uveal melanomas, blue nevi, and meningeal melanoma	Initiation - Associated with DNA repair; Uveal melanoma and familial melanomas

Some may be directly related to UV radiation instigation and others may be due to endogenous or exogenous factors. The formation of melanoma has been simplified through a schematic of melanocyte → nevi → dysplastic nevi → melanoma in situ → invasive melanoma. This step-by-step progression though does not tell the whole story. It has been demonstrated that different melanoma types will skip over specific steps of this schematic[4,8] (Figure 1).

Figure 1: Melanomagenesis



A multi-step carcinogenic pathway is underlying melanoma development, driven by numerous genetic events. An example of this is mucosal melanoma, in which there are no known intermediate steps from normal melanocytes → melanoma in situ. Even different versions of cutaneous melanoma have been documented to skip phases of development.[4] Presence of nevi does not correlate directly to risk factors for the development of cutaneous melanomas. Understanding and identifying precursor lesions, tumor microenvironment, and endogenous host factors may lead to more insight into melanomagenesis.

Identifying genetic and epigenetic players in melanomagenesis will assist in the progress toward prevention, detection, and treatment. Hayward et al.[9] published a genomic landscape on cutaneous, acral, and mucosal subtypes. For cutaneous melanoma BRAF, CDKN2A, NRAS, and TP53 were found to be the most mutated genes. Variants of genetic players in cutaneous melanoma may influence the ratio of pheomelanin to eumelanin, causing an increase in the vulnerability of the cells.[4] Acral melanoma showed c-KIT, BRAF, NRAS, and NF1 most commonly mutated, while c-KIT and SF3B1 dominated mucosal melanoma. It is interesting to note that melanoma has the highest mutational load of any cancer. Acral and mucosal melanoma are genetically differentiated from cutaneous melanomas not only by the genes mapped to the diseases, but also in the fact their mutational burden is lower. Characteristics of uveal melanoma show yet another genetic mutational pattern with GNAQ and SF3B1 being prominent.[10] In addition to coding mutations found, Hayward et al. also discovered non-coding mutations as significant due

to their potential genetic driver capabilities.[9] Other than an alphabet soup of genetic players, these insights give researchers new clinical pathways. Understanding the how and why of melanoma presents novel ways to attack the progression of melanoma.

While the majority of melanomas are caused by exogenous factors and exposures, some are hereditary. Predisposing genetic mutations include CDKN2A, CDK4, TERT, MITF, POT1, PTEN, and BAP1. For example, mutations in germline BAP1 lead to BAP1 tumor syndrome and have been shown to be related to increased risk of cutaneous, uveal, and internal melanomas.[11] This BAP1 familial syndrome has been linked to other cancers as well such as mesothelioma, breast cancer, renal cell carcinoma, and prostate cancer.[12] Early-age onset of melanoma may indicate a germline mutation and be related to POT1 or MITF variants. Not enough data has been accumulated due to the rarity of the hereditary melanoma syndromes, thus intensive study is needed to gain conclusive understanding of genetic instigators.

According to Bastian[8] the taxonomy of melanoma should be divided into two different categories: epithelium associated and non-epithelium associated (Table I). The dependence of epithelium-associated melanomas on UV radiation varies with a high correlation for chronically sun-damaged tissues to lower to no correlation for acral and mucosal melanoma. Melanomas, whether epithelium-associated or not, show multiple different classification in clinical and/or histopathological presentation, role of UV radiation, ethnicity, age of onset, germ and somatic alterations, and predicted site for metastatic spread.

Division of melanoma research has mainly focused on site of origin.[13] Cutaneous melanomas appear on the non-glabrous skin; acral melanoma originates on the glabrous skin of the palms, soles, and nail beds; mucosal melanoma arises in the internal or mucosal lining of the body; and uveal melanoma develops from the melanocytes of the choroid plexus, ciliary body, and iris of the eye[10] (Table I). Shain and Bastian[4] noted that the distinct subtypes of melanoma present different vulnerabilities that may be exploited for clinical gain, such as new prevention, detection, and/or treatment methodologies.

Progression of melanoma is highly dependent on the subtype. From initiation to development and spread, melanoma cells become more genetically complex, leading to opportunistic ability to spread to different locations. The genetic diversity of melanoma subtypes may relate to the preferred location of metastatic tumor locales. For example,[14] superficial spreading melanoma and nodular melanoma frequently spread to the lung and brain. Acral and mucosal melanomas have been found to preferentially metastasize to the bone while uveal melanomas spread to the liver.[15] These differences may demonstrate the need to understand not only the genetic diversity of melanoma subtypes, but to also clearly demonstrate how the tumor microenvironment influences the spread of the disease.

For cutaneous melanoma, it is thought that it spreads to both local and distal sites with the same probability and at the same time. Tumor cells can disperse via both the vascular system and the lymphatic system. Some evidence suggests that regional nodes may not play the boundary role. Positive nodes in melanoma may serve as only biomarkers that metastatic spread has already occurred.[4] Often the primary tumor cannot be identified and only the metastatic tumors are found. Sentinel lymph node dissection in melanoma does not improve long-term survival as it may in other cancers, although it serves as a prognostic marker.[16] (See Section VII: Dormancy, Recurrence, and Metastatic Disease).

For all melanoma subtypes, metastatic progression causes the majority of deaths. Multiple factors play a role in metastatic melanoma, including subtype, thickness of primary tumor, sex, age, and ulceration. Tumor dormancy, appears to play an important role in patient outcomes. Key characteristics of dormancy

are epigenetic drivers. Mapping these is expected to pave new ideas for therapy, independent of genetic drivers. Often, the time between the primary tumor treatment and appearance of metastatic disease may be years. Recurrence is usually not at the primary tumor site, but at a distal region.[16] The consequences of a protracted disease state leaves an open door for researchers and clinicians to exploit new avenues to fight melanoma.

Understanding the process of melanomagenesis holds the keys toward prevention beyond sunscreen, advances in screening and diagnostic tools, prognostic predictors, better treatment, and survivorship.

SECTION II: **Melanoma Incidence and Mortality Rates**

In the US, melanoma is the most diagnosed cancer among men and women ages 25-29 years and the fifth most diagnosed cancer in men and women overall.[17] Incidence rates for cutaneous melanoma have increased over the last several decades[18] with an estimated 100,350 people were diagnosed with new melanomas in 2020.[19] However; mortality rates have recently been on the decline due to earlier diagnosis and advancements in treatments for melanoma.[20] Gender, age, geographic region, and racial/ethnic differences all play a large role in determining incidence of melanoma. Indeed, melanoma is 20 times more common in white-skinned than black-skinned people,[21] correlating with a lower amount of photo-protective melanin (eumelanin) present in lighter skin.[22-24] Overall, the lifetime risk of acquiring melanoma is about 2.5% (1 in 40) for whites, 0.1% (1 in 1,000) for blacks, 0.5% (1 in 200) for Hispanics, and 1 in 100,000 for Asian/Pacific Islanders populations.[21] While adolescent and young adult (AYA) women appear to be more susceptible to melanoma than AYA men, men over age 40 are more likely to be diagnosed with melanoma than same-aged women. Incidence in general is drastically increased with age, generally peaking after the sixth decade of life. By age 65, incidence rates in men are double those in women; at age 80, rates in men are triple that of women at the same age. In the US, it is predicted that annual new cases of melanoma will continue to increase in the white demographic, primarily due to population growth and aging.

Melanoma worldwide incidence has increased over the last several decades. Globally, the highest incidence is found in Australia and New Zealand, with rates twice as high as in North America. Contributing factors include proximity to the equator, reduced ozone layer, and an abundance of primarily fair-skinned populations that are more susceptible in these countries.[25-30] Thanks largely in part to the development and implementation of an extensive skin cancer screening program, average tumor depth or stage of melanoma at time of diagnosis has decreased in Australia, shedding light on the importance of detection. Some investigators have gone as far as to suggest that increased incidence is due in part to improved screening and diagnostic tools, and better access to healthcare; however, several studies have indicated there is a true increase in melanoma incidence.[31]

Higher incidence of melanoma has also been associated with occupational sun exposure accompanying Service in the military. Studies have found that Service in tropical environments close to the equator, along with a lack of preventative measures or compliance with preventative measures, contribute to higher incidence of skin cancer among US military and Veterans. A review in 2010 of tumor registry from the DOD and the National Cancer Institutes found that people who had served in the military and were 45 years old or older had a significantly higher melanoma incidence rate compared to the general US population.[32] Melanoma incidence is increasing among active duty Service members, with the greatest incidence rates in the Air Force, Navy, and the Marines, with the Air Force presenting with the highest rate of incidence among the branches. Looking forward, this raises concerns for the more than 3 million Service members deployed from 2001 to 2014 who participated in Enduring Freedom/Operation Iraqi Freedom missions in

Afghanistan and Iraq. A revealing 2015 study reported less than 13% of soldiers surveyed used protective measures, such as sunscreen, on a regular basis during deployment.[33,34]

Survival rates of melanoma vary widely based on type (Table I) and stage at the time of diagnosis. While some types of melanoma such as superficial spreading melanoma exhibit favorable 5-year and/or longer-term survival rates, other prognoses are quite bleak. For instance, in most cases of pediatric leptomeningeal disease, survival is generally measured in weeks to a few months. Interestingly, even differing anatomic regions of a single subtype of melanoma (e.g., mucosal) display varying outcomes, highlighting the need for improved understanding of these less common variants.

SECTION III: **Risk Factors**

Melanoma risk factors are environmentally and genetically based, with overall risk depending on interactions between both sources. Age, sex, UV exposure, body characteristics like skin type or hair color, personal history (medical and dietary), chemical or ionizing radiation exposure, and genetic susceptibility are all known risk factors that can contribute to developing melanoma.[35] While some risk factors, such as exposure to UV radiation, can be easily prevented through modifying behavior, others represent inherent biological risks.

There is a direct correlation of melanoma risk with age and sex.[25, 36] Melanoma mostly affects younger to middle-aged people, with the average age at diagnosis of 57 years. While incidence rates are higher in women than in men before age 50 (0.6%), by age 65, the male population experiences a doubling in melanoma incidence rates compared to women. Strikingly, by age 80, the incidence rate of melanoma in men is triple that in females.[37] Both age and sex influence melanoma development, as well as response to therapy; however, the specific cellular and molecular processes dictating these variances are not yet fully understood.[38] It is evident that the process of aging and accumulation of cellular mutations can lead to tumor progression.[39] Many processes altered by age, such as intracellular communication, proteostasis, mitochondrial dysfunction, cellular senescence, and tumor microenvironment, are significant factors in cancer development.[38] Indeed, even changes in stroma and the extracellular matrix can influence melanoma progression.[40-41]

Determining the exact biological sex differences influencing melanoma risk and survival is controversial, with many studies offering conflicting results.[42-44] Melanoma incidence in sexes may vary due to hormone levels such as androgen and estrogen,[45-46] or differences in handling oxidative stress.[41] Post-menopausal women exhibit an increased rate of melanoma survival in multiple studies,[47-53] while a few studies present directly conflicting results.[54-56] In another study, increased risk of melanoma correlates with male obesity,[57] signifying that host body features are also influential factors. There is also evidence of an association between melanoma and prostate cancer in males.[45, 57-58] Therefore, sex and age remain interrelated risk factors for melanoma.

The chief environmental risk factor for melanoma is exposure to UV radiation from the sun[59-61] and artificial light,[62] however, UV is also one of the most easily preventable risks. Elements such as geographic location, sun sensitivity or inability to tan, sunburn history,[62] and melanin type further influence risk of UV exposure. Populations living near the equator experience an increased risk of UV from the sun. Other environmental characteristics, such as atmospheric absorption of UV, UV type A or B, latitude and altitude, amount of cloud cover, and seasonal effects also influence UV exposure.[25]

Risk factors for development of cutaneous melanoma in Service members are significant. Due to the deployment to areas of high solar radiation, both active duty and Veterans are at increased risk.[63] Additionally, the members of the military may be exposed to other carcinogens, such as polychlorinated biphenyls (found in older military equipment, i.e., Navy vessels[64]) or jet exhaust and more intense radiation (i.e., pilots in the Air Force). These risk factors have long-term impact on Veterans as well. Evaluations of medical charts for Veterans at the Minneapolis VA Hospital showed 37% of melanomas found were not the reason for the initial consult.[65] Another study showed that a majority of Veterans reported inadequate access to sun protective equipment during their missions despite spending more than 4 hours a day in bright sun.[66]

Sunburn history, including total number of burns, age at time of sunburn, and length of UV exposure, is another feature that contributes to risk of developing melanoma.[60, 62] In fact, a history of sunburns experienced during childhood is associated with higher risk of developing melanoma.[62] Several studies have discovered an association with intermittent sun exposure and increased melanoma risk.[60, 62]

While risk factors such as UV exposure are more obviously associated with development of cutaneous melanoma, rare melanoma subtypes have unique or relatively unexplored risk factors. Ocular melanomas, such as uveal and conjunctival melanoma, are inherently different when compared to cutaneous melanomas. A review of meta-analyses of risk factors by Nayman et al.[67] determined the following nine risk factors for uveal melanoma: atypical cutaneous nevi, common cutaneous nevi, iris nevi, light eye color (blue, green, grey), fair skin color, cutaneous freckles, propensity to sunburn, welding, and occupational cooking.[31] Light iris color, fair skin, freckles, nevi on the upper arms, burns to the eyes, use of sunlamps, ability to tan, and working outside for 4 or more hours per day were also determined as risk factors from meta-analyses.[68-70] Regarding conjunctival melanoma, a lack of tumor pigmentation increases risk of metastases and reduces overall survival rate.[71-72] It is apparent that, although there are a handful of similar risk factors between ocular and cutaneous melanomas, these risk factors and pathogenesis of uveal and conjunctival melanomas must be further elucidated.

Acral lentiginous melanoma (ALM) is a rare variant of melanoma most common in individuals with darker skin types such as Asians, African Americans, and Middle-Easterners.[73] While other forms of melanoma tend to develop on light skinned individuals on areas exposed to UV, ALM lesions appear on sun-protected areas such as the palms, soles of the feet, and under nail beds. ALM is also most commonly diagnosed in the seventh decade of life, suggesting that age plays a major role in development.[74] Expression of the c-KIT gene is significantly associated with ALM progression.[75] A mutation in c-KIT causing excessive differentiation and proliferation of melanocytes appears to play a large role. However, the risk factors for this rare subtype of melanoma are still unclear and require more investigation.[73]

Another unique subtype of melanomas includes those which develop in many sun-protected areas in the surface of mucosal linings throughout the body. Exposure to UV-R is not an apparent risk factor for these melanomas. Similarly with ALM, c-KIT mutations are associated with mucosal melanomas, and again, this rare subtype of melanoma is understudied when compared to cutaneous melanomas. Specific details related to risk factors and pathogenesis of mucosal melanomas remain undiscovered, mainly due to rarity and later stage diagnoses common with this subtype.[76-77]

SECTION IV: Prevention

In fiscal year 2019 (FY19), when the Peer Reviewed Melanoma Research Program (MRP) under the DOD, the Congressionally Directed Medical Research Programs held a Stakeholders meeting to review the

clinical and research landscape, the outcomes of the discussions concluded that the field sorely lacked understanding and advanced methods of prevention. To minimize incidence and increase survival of melanoma, the role of prevention must evolve and acknowledge each step of the disease, from primary and secondary prevention, to detection and monitoring, initiation and progression, and reemergence from tumor dormancy. Each juncture along the disease progression represents an important opportunity to develop preventative treatments to halt the initiation and spread of melanoma. In response to these highly significant gaps in care and research, the MRP issued a Challenge Statement that has been incorporated into the investment strategy and focus areas of the program.

FY20 MRP Challenge Statement: The MRP challenges the research community to redefine the concept of prevention. Melanomagenesis is a multi-step process initiating from normal melanocytes to dysplasia, through the development of melanoma and metastasis. A new paradigm of prevention may include detection, monitoring, and stopping the initiation of dysplasia, halting the progress to malignancy, blocking micro-metastases, or preventing emergence from tumor dormancy. The MRP acknowledges that each step along the disease process from initiation to metastasis is an opportunity to detect, monitor, and impede progression and to effect a cure. The MRP challenges the research community to prevent melanoma earlier in the disease cycle, thus preventing metastasis. The melanoma clinical, research, and patient community traditionally view prevention as the use of sunscreen/blockers to protect the melanocyte from harmful UV radiation. The MRP recognizes the usefulness of this strategy, while tasking the research community to redefine prevention to include the entire melanomagenesis process. Disease progression in melanomagenesis may not be as depicted linear and straightforward, but still may be halted and prevented to affect better outcomes for all melanoma patients. This is especially critical in rare subtypes of melanoma where traditional sunscreen blockers are not applicable. Rare melanoma subtypes (i.e., acral, uveal, and mucosal) may not be initiated by exposure to ultraviolet radiation like cutaneous melanoma. Taken together, the MRP looks to shift the paradigm of prevention of all types of melanoma by investing in research studies focused on eliminating the progress of this deadly disease, whether it is cutaneous melanoma or a rare subtype.

Standard prevention of melanoma, especially rare subtypes, is still in its infancy and as such, the following prevention methods described pertain largely to cutaneous melanoma. Basic prevention methods for melanoma include the time-honored use of sunscreen, avoidance of prolonged exposure to direct sunlight, and/or specialized UV protective clothing. The two chief varieties of sunscreen include mineral and chemical sunscreens. Both options create a barrier to help protect the skin by either absorbing (chemical) or reflecting (mineral) harmful UV radiation.[78-79] Currently, the U.S. Food and Drug Administration (FDA) approves of approximately 16 active ingredients in sunscreens, including the inorganic agents titanium dioxide (TiO_2) and zinc oxide (ZnO).[78] Many sunscreens are composed of a mixture of these active ingredients in order to provide safer, improved protection.[80] Sunscreen products are now available in a variety of safe and effective forms, including lotions, creams, sprays, oils, gels, butters, ointments, pastes, and sticks.[81] They also come in a variety of sun protection factor (SPF) values, with higher values (up to 50 SPF) providing greater protection against UVB radiation and sunburn. The FDA also recommends use of a broad spectrum sunscreen that is proven effective against both UVA and UVB radiation. Overall, a broad spectrum sunscreen with an SPF value of 15 or higher is recommended.[1] Reapplying sunscreen at least every 2 hours is also suggested, as the product can eventually be diluted or wash off due to sweat or water. It has been shown, however, by Premi et al.,[82] that exposure to UV damaging radiation and the resulting oxidative cascade does not stop immediately after exposure. In other words, “DNA damage continues in the dark.” Therefore, it is advised that, once sun exposure and possible burn occurs, sunscreen is to be continually applied, even after the exposure. The sunscreen must contain ethyl sorbate in order to stop the

continued DNA damage.[82] The continued DNA damage was found to be associated with a high level of pheomelanin (found in fair skin, red hair people). The oxidative damage that occurs (see Section I: Melanomagenesis) presents a novel avenue to pursue for prevention of initiation of melanoma, thus moving beyond the traditional prevention strategies.

Melanoma prevention is generally divided into two areas: primary prevention and secondary prevention. Primary prevention includes measures such as avoiding UV radiation by utilizing sunscreen or donning protective clothing or eyewear, and therapeutic or chemopreventative methods. According to Chhabra et al.,[83] chemoprevention may be divided into three categories (1) agents that prevent the development a primary melanoma; (2) agents that aim to stop the progression of a pre-malignant lesion to a malignant lesion; and (3) agents that prevent the recurrence of melanoma. No agents are approved by the FDA for chemoprevention of melanoma. In fact, this area of study has been deemed under-researched and has been acknowledged as a gap in understanding of melanoma progression and treatment.[83]

Current chemoprevention utilizes agents that may manipulate growth factors, influence the activity of proto-oncogenes, and induce anti-inflammatory responses. The protective effect of vitamins has been studied extensively, including roles of vitamins A, C, D, B3, E, and K.[84] Results have been mixed.[8] This has been attributed to lack of information on the vitamins' impact on anti-tumor pathways. Topical retinoid has shown some promise, with reports demonstrating improvement of dysplastic nevi.[85] The process of cutaneous melanomagenesis relies heavily on the production of radicals that lead to DNA damage. Studies of antioxidants such as vitamin C have shown they provide little protection.[86] This may be due to the issue of chemiexictation of melanin derivatives that linger and cause DNA damage long after the initial UV radiation exposure.[82]

Other dietary supplements have been implicated in the cancer chemoprevention. Resveratrol, found in red grapes (red wine) and peanuts shows promise, as studies demonstrated that it inhibits the initiation and progression of skin cancers. Laboratory studies of topical Resveratrol substantiated its chemopreventative properties, especially with melanoma. Unfortunately, due to its short half-life, preclinical studies have been less than encouraging.[83]

Bioavailability and short half-life have plagued other natural supplements for cancer prevention as well. Curcumin from turmeric has been floated as an ideal candidate for chemoprevention for melanoma. Studies have demonstrated that curcumin may inhibit epithelial-mesenchymal transition (EMT) and thus, stop imitation and progression of melanoma metastasis. However the low bioavailability of oral curcumin has been a stumbling block in its usage.[87] Analogues are under development. The molecular mechanism of curcumin and its analogues are dependent on the JAK2/STAT3 pathway as well as miRNAs that regulate cell cycle and apoptosis. Another dietary agent is Fisetin, a flavonoid that has been investigated over the years as a potential preventative and treatment. Fisetin, like curcumin inhibits the transition of melanoma cells from the epithelium through the mesenchymal layer.[88] The targeting of the YB1/RSK and ERK pathways highlight its potential as an intervention along the pathogenesis of melanoma. It has also been promoted as a possible treatment to stop the spread of uveal melanoma.[89] Studies are still premature and are not supported by strong clinical data.

Other prevention candidates include non-steroidal anti-inflammatory drugs (NSAIDs). Aspirin and other NSAIDs have been in the public consciousness as potential chemopreventative agents for cancer for decades. Clinical studies utilizing NSAIDs as a melanoma preventive yielded only inconsistent results.[90] NSAIDs act as an inhibitor on the COX2 pathway, thus limiting the exposure of cells to prostaglandins that

may promote cellular proliferation, invasion, and metastasis. Using a COX2 inhibitor is attractive because of these characteristics. Another attractive feature of COX2 inhibitors in melanoma prevention is that it is not expressed in benign nevi. This differential expression helps to target the NSAIDs to the melanoma cells.[90] Further clinical research is needed to clarify the usefulness of NSAIDs not only as preventatives, but also as a supplementary treatment.[91]

Other unexpected candidates include statins. Some studies indicate that statins have an anti-proliferation effect and that they may regulate the RAS pathway. Current clinical studies have been unable to demonstrate any advantage in preventing melanoma as a side effect consequence when statins have been prescribed to patients for cardiovascular issues.[83] No decrease in melanoma incidence has been found in this study population.

Prevention is considered to be a leading knowledge gap in melanoma research and clinical practice. The redefinition of prevention beyond just sunscreen also includes the idea that stopping melanoma progression through the life cycle will lead to better outcomes. Prevention is key to ending the toll of melanoma on patients.

SECTION V: Detection, Diagnosis, and Prognosis

The heterogeneity of melanoma plays an important role in the detection, diagnosis, and prognosis of the disease. Histological features of melanoma were first used to classify it into superficial spreading melanoma, lentigo maligna melanoma, and nodular melanoma.[1] Diagnostic features were mainly macroscopic before the 1980s, with large, ulcerated, and fungating being the most prominent visual elements used.[2] These recognizable features indicate an advanced disease and thus, are far too late for successful therapeutics. In the mid-1980s, Rigel et al.[92] devised the standard and popular ABCD system (Asymmetry, Border Irregularity, Color Variegation, Diameter >6mm). In addition to identifying the differences of cutaneous melanoma, Wallace Clark also created a system to evaluate the depth of invasion of melanoma (Table III).

Table III Clark Levels of Invasion of Cutaneous Melanoma

LEVEL 1	Cancer cells confined to epidermis
LEVEL 2	Breakthrough of cancer cells into the papillary dermis
LEVEL 3	Expansion of cancer cells through the papillary dermis
LEVEL 4	Cancers cells invade the reticular dermis
LEVEL 5	Cancer cells invade the subcutaneous fat layer

One of the best indicators of prognosis is the thickness of the primary melanoma. Alexander Breslow's system based the classification of melanoma on the measured thickness of the tumor and not the anatomical compartments of invasion like Clark's method (Table IV).

Table IV: Breslow Classification of Cutaneous Melanoma

Stage I	Primary melanoma tumor ≤ 0.75 mm
Stage II	Primary melanoma tumor 0.76-1.5 mm
Stage III	Primary melanoma tumor 1.51-2.25 mm
Stage IV	Primary melanoma tumor 2.26 – 3.0 mm
Stage V	Primary melanoma tumor > 3.0 mm

It has been observed that patients with thinner melanomas have a better prognosis than patients with thicker, deeper primary tumors.[1] While these different classification systems have their positive values, the need for better and more precise methods for diagnosis is paramount to increasing the long-term outcomes for cutaneous melanoma patients.

One of the main constraints on detection of melanoma happens at the primary care level. Most family doctors are not well versed in the detection of melanoma, nor are they able to do a total body skin examination (TBSE) of patients who are at high risk with any reliability.[93] A call to action in 2014 by the US Surgeon General to increase TBSE has not elicited the same response in public health as more well-known screening protocols such as mammograms and colonoscopies for breast cancer and colorectal cancer, respectively. Patient demand, not physician orders, is the main driver of TBSE. Unfortunately, the methodology and consistency in which TBSE is performed by both general physicians and dermatologists remains questionable.

One of the hallmark methods to visualize the deeper epidermis and thus, clarify the thickness of a potential cutaneous lesions, is the use of dermoscopy. For topical tumors, the sensitivity of dermoscopy as compared to the review by the naked eye showed up to 92% sensitivity and 80% specificity.[93] Dermatologists are superior in their evaluations in comparison to family doctors using the dermoscope. Rare variants (Table I) of epidermal melanoma may be missed or misdiagnosed by both dermatologists and pathologists. Decision-making tools, more advanced imaging, and better biomarkers are urgently needed to facilitate better detection and diagnoses of melanoma and rarer variants.[94]

Further development of melanoma diagnostic tools would offer a better outlook for melanoma patients and those at risk if their disease is caught earlier. Multiple advances may lead to better methods when studied against TBSE, dermoscopy, and total body photography.[93] All of these methods rely on the inspection of the skin lesion by eye and/or subjective evaluation. Even biopsies of lesions do not guarantee definitive diagnosis, as it has been reported that pathologists will disagree on the malignancy of a nevi with up to 15% discordance.[95]

Different imaging systems have arisen in the last decade, including sequential digital dermoscopic imaging (SDDI) and integrated total body photography with SDDI. Evidence supporting the use of SDDI for detection and diagnosis remains weak, with some studies showing an advantage of the SDDI technology that utilizes sequential capture of dermoscopic images over time, while others report no significant benefit.[3] All dermoscopic imaging relies on diagnostic algorithms to differentiate the malignant from the benign lesion. Evaluation includes using the ABCD rules, the color, architecture, symmetry, and homogeneity

(CASH), the 7-point checklist (weighted), and the Menzies method.[92, 96-97] A comparison of methods by Carrera et al.[97] demonstrated both the strengths and weakness of these and other dermoscopic methodologies. This study, though, did not identify the best method for differentiation and, in fact, supported further studies via "*crowd-sourcing and collective intelligence approaches"* and concluded that "*these efforts will lead to a unified dermoscopic algorithm, automated detection of criteria, and clinical decision support systems that facilitate population-based melanoma screening efforts."*

Emerging technologies for melanoma detection and diagnosis are based on spectral analysis, laser microscopy, tomography, ultrasound technology, and bioimpedance.[92] None of these technologies or any others present a sensitivity and specificity without some disadvantages for the diagnosis of cutaneous melanoma, especially since there are multiple types of cutaneous melanoma that offer different characteristics and qualities that may not be accounted for in the methodologies. Thus, the right technology for the lesion is critical in diagnoses.

Continued issues with proper diagnoses of melanoma must be addressed with easily accessible technology. It is important to note that increased use of advanced technology may lead to increases in excisions of lesions that may have never advanced. Therefore, the use of computer-aided decision making regarding potentially malignant lesions must be weighed against the more traditional diagnostic methodology. Additional meta-analyses of the developing technologies will support and balance how technology will be utilized against traditional evaluations.

Beyond the visualization of lesions, biomarkers have been posited as a method for not only detection of melanoma, but diagnostic and prognostic tools. An early method using mRNA patterns called tape stripping has been employed in the clinic. However, cytological examination of mRNA patterns only resulted in low sensitivities and specificities.[92] Circulating mRNA as biomarkers are currently under study.[98]

These diagnostic challenges for more common types of melanoma continue to delay needed treatment. For rare subtypes of melanoma, a crisis exists for the clinic and patient outcomes. For example, the rare subtype of acral melanoma is a form of malignant melanoma prevalent in persons of color. It is considered a subtype of cutaneous melanoma because it originates from melanocytes of the epidermis.[8] Many acral melanoma lesions are missed because of the difficulty in differentiating them from benign lesions of the palms, soles, and nailbeds. Accounting for 2%-3% of melanomas in the United States, acral melanomas have a significantly worse prognosis and outcome due mainly to the lateness of diagnosis and treatment options.[99] Conventional diagnostic criteria for cutaneous melanoma does not relate to acral lesions due to unusual features such as band pigmentation, although nearly a third of acral lesions fail to show this diagnostic pattern.[100] Criscito and Stein[100] reviewed the best diagnostic tools for detection of acral melanoma, publishing practicing points, including: "*dermoscopy provides an effective noninvasive modality to differentiate benign acral melanocytic nevi,"* and "*measurement of the maximum diameter of acral lesions… to determine management."* It is important to note that acral melanoma has a high rate of recurrence and a worse prognosis than other cutaneous melanomas. This has been attributed not only to the different genetic patterns of expression, but also to the delayed diagnosis of the disease. The lack of early diagnosis may be due in part to the lack of education for primary care physicians and dermatologist for this rare subtype.

Another epidermal variant, mucosal melanoma, is a disease that appears in mucosal linings of the epithelium including the vulva, vagina, rectum/anus, mouth, and sinus-nasal cavity. It is a rare aggressive

subtype of melanoma, and overall survival is 25% for 5 years (regardless of stage).[101] Due to its rarity, the diagnosis and detection of mucosal melanoma is fraught with issues of misdiagnosis. Staging of this rare melanoma with Breslow depth analysis garners no useful information.[94] The less visualized areas where mucosal melanoma appears play a significant role in the late diagnosis of the disease. Biomarkers such as circulating tumor markers or imaging markers may offer a potential avenue of earlier detection. Distinct molecular patterns of mucosal melanoma may offer hope in being able to employ diagnostic markers. Genetic mutations and causes of mucosal melanoma are still under study. The most frequently mutated genes for mucosal melanoma are NRAS, BRAF, NF1, KIT, SF3B1, and TP53,[102] thus offering targets for detection and treatment. Mucosal melanoma presents with lower point mutations and higher structural chromosomal aberrations.[102] As of yet, researchers have not been able to exploit these differences for detection or treatment.

Uveal melanoma is the most commonly diagnosed cancer of the eye. These melanomas are not of the epithelium but of the choroid, ciliary body, and iris of the eye. For diagnostic measures, patients complain to their doctors of blurred/distorted vision or loss of vision. Methods (e.g., visual examination, fluorescein angiography, ocular echography) should be able to differentially diagnose benign nevi of the eye from malignant ones. Ophthalmologists may use clinical features (sub-retinal fluid, orange pigment, fundus growth) to distinguish the malignant from the benign.[103] It has been noted that biopsy is not required and that the Collaborative Ocular Melanoma Study[104] presented a 99% diagnostic accuracy due to typical features of uveal melanoma (e.g., low internal reflectivity, “quiet zone” on ultrasound, domed, collar stud, or mushroom shape, and larger dimensions). These methodologies have not changed in over 20 years. Some tumors may cause different symptoms such as flickering/flashing images due to retinal detachment.[103] The retinal detachment is often treated with removal of the vitreous humor prior to the cause (melanoma) being identified. The vitreous humor is replaced by a silicone oil mixture in the eye, but this causes complications on imaging the ultimate culprit–the melanoma. One imaging technique for uveal melanoma is ultrasound. If the vitreous humor has been replaced by silicone oil mixture the scans are not reliable, therefore Jaarsma-Coes et al.[105] reported on the use of MRI to diagnose this unique uveal melanoma patient population. Predicting whether a uveal melanoma will spread to distant sites is still an under-researched area.

Prognostic factors to predict patient outcomes are highly dependent on the type of melanoma as well as the thickness, invasion, and genetic profile. While the Clark and Breslow methodologies may be used to first classify the tumor, the American Joint Committee on Cancer (AJCC) devised the Tumor-Node-Metastasis (TNM) staging system for cancers in 1998, and it has been used to modify how melanomas are staged and treated.[106]

It has been noted previously that areas of the epithelium (skin) chronically exposed to the sun have the highest mutational burden when developing cancer, especially melanoma. The UV radiation mutation is the signature C>T transition.[13] This leads to the known genetic profile of cutaneous melanoma (Table II). The 5-year survival rate for skin melanomas overall, according to Surveillance, Epidemiology, and End Results (SEER) data collected 2009-2015, is shown in Table Va. The SEER data is aggregated data and does not consider the different types of cutaneous melanoma. Additionally, the staging of cutaneous melanoma is complex and does not adhere well to the simple Stage I-IV TNM as other cancers do.

Table Va: SEER DATA: 5-Year Survival Rates for Cutaneous Melanoma

SEER*	5-Year Relative Survival Rate
Localized	99%
Regional	65%
Distant	25%

* https://www.cancer.org/cancer/melanoma-skin-cancer/detection-diagnosis-staging/survival-rates-for-melanoma-skin-cancer-by-stage.html

Other variants of melanoma arising in areas of the body other than the skin will have different survival rates. Uveal has a slightly lower survival rate over all locations and SEER stage (Table Vb).

Table Vb: SEER DATA: 5-Year Survival Rates for Uveal Melanoma

SEER*	5-Year Relative Survival Rate
Localized	85%
Regional	71%
Distant	13%

*https://www.cancer.org/cancer/eye-cancer/detection-diagnosis-staging/survival-rates.html

For mucosal melanoma the survival rates are categorized by primary tumor site (Table VI).

Table VI: 5-Year Survival Rates for Mucosal Melanoma by Primary Tumor Site

Primary Tumor Site*	5-Year Relative Survival Rate
Head and Neck	12%-30%
Vulva	24%-77%
Vaginal	5%-25%
Anorectal	20%

* https://www.dermnetnz.org/topics/mucosal-melanoma

Acral Melanoma has a lower survival rate than other epithelium-based melanomas (Table VII), with a 5-year survival rate of 67% (African Americans), 72% (Hispanic White), 77% (Asian/Pacific Islanders), and 84% (Non-Hispanic Whites) according to Huang et al., based on SEER data from 2006-2015.[107]

Table VII: Five Year Survival Rates for Acral Melanoma by Race-Based on SEER Data (2006-2015)

Race	5-Year Relative Survival Rate
Non-Hispanic Whites	84%
Asian/Pacific Islanders	77%
Hispanic Whites	72%
African Americans	67%

Prognosis of cutaneous melanoma based on staging and classic thickness measures of the primary tumor may lead lower risk patients to erroneously believe they have little to worry about regarding relapse with increase of stage and severity of disease. In fact, as published in a study from Australia, more than two-

thirds of patients who die from cutaneous melanomas were originally Stage I or Stage II.[108] Identifying those Stage I and/or Stage II patients at higher risk for recurrence is critical for prognostic understanding of the disease as well as decision-making for treatment and surveillance.

It is clear that the genetic drivers and biomarkers for prognosis and therapeutics is important for all subtypes of melanoma, but especially for those rarer variants. The complexity of instigators of melanoma and the resulting genetic burden play roles that are not yet understood or exploited. While treatment options are advancing for cutaneous melanomas, other melanomas are being left behind. Research needs to advance to serve these variants as well as resistant and recurrent melanomas.

SECTION VI: Treatment

Surgery

For patients with melanoma, the primary surgical recommendation is wide excision and sentinel lymph node (SLN) biopsy. In some cases, complete lymph node dissection may be necessary.[109] For the treatment of lentigo maligna melanoma (also known as melanoma in situ), alternate therapies may be considered such as off-label use of imiquimod, radiation therapy, or observation.[110]

Wide excision is defined as surgery to remove the melanoma and a margin of healthy tissue surrounding the tumor. Once melanoma has been confirmed histologically, a wider and deeper excision is necessary to reduce the risk of recurrence. A surgical margin of 1-2 cm is recommended based on thickness, in order to achieve histologically negative margins.[111] The recommendation for excision is that it extends to the level of muscle fascia or deep adipose tissue depending on the location of the melanoma.[110] Wide excision is the main treatment option for most early stage cutaneous melanomas, as well as acral melanomas on the dorsal and volar surfaces of the hands and feet and mucosal melanomas. Enucleation (removal of the eye) is an option in cases of uveal melanoma where the tumor is very large or if radiation could lead to severe visual complications.[14]

Mohs micrographic surgery (MMS) is used to treat the earliest stage of melanoma, lentigo malignant melanoma. Compared to wide excision, MMS offers the benefit of tissue conservation, margin control, and histological assessment in real time.[111] During this procedure, the visible melanoma is removed along with a small amount of normal looking skin. The surgeon analyzes the removed tissue microscopically for cancerous cells. The surgeon continues to remove thin layers of skin for analysis until no cancer is seen.[112] In a study of 1,072 patients, 86.0% of lesions were excised with margins of 6mm, and 98.9% of lesions excised with 9mm margins. With these margins, only 0.3% patients experienced recurrence.[113] Immunostaining for melanoma-associated antigen recognized by T cells (MART-1) during histological analysis has demonstrated efficacy. In a retrospective study evaluating 1,982 patients who underwent MMS excision followed by MART-1 immunostaining, the 5-year recurrence rate was 0.59% and survival rate was 98.53%.[114]

A SLN biopsy may be recommended to determine if melanoma has spread beyond the primary tumor. The SLNs are the predicted initial site of metastasis of the primary tumor. The false negative rate for SLN biopsy is 15%-25%.[109,115] For patients with a positive SLN biopsy, complete lymph node dissection (CLND) is recommended, where all the cancer draining lymph nodes are removed. However, a large multi-center clinical trial (MSLT-2) compared outcomes of patients who underwent CLND following a positive SLN biopsy with follow-up observations and found that, while CLND did control metastasis, there was no difference in overall survival.[116] Once melanoma has metastasized, surgery alone is unlikely to be curative.

Removing some visible metastasis may alleviate symptoms and improve quality of life. However, for metastatic disease, systemic treatments such as immunotherapy or chemotherapy are recommended treatments.[117,118]

In very rare cases, amputation of digits or appendages may be necessary. Acral melanomas on the nail fold, subungual region, or digits have traditionally been managed with digit amputation.[119] Amputation may also be considered if the melanoma has grown back after multiple surgeries, has spread extensively to nearby tissues, has caused loss of function in that body part, or causes pain that cannot be controlled.[117,120] For larger tumors, surgeons may need to consider reconstructive techniques, which involve grafting skin from one area of the body onto the affected area.

Radiation

Radiation therapy is the use of high-energy particles such as X-rays, gamma rays, electron beams, or protons to target tumors by damaging their cellular DNA. External beam radiation is a localized therapy that directs radiation in the form of photons, protons, or electrons at tumors. For cases of melanoma in situ, radiation is the best option if surgical removal of the lesion is not possible. Retrospective analysis suggests that there is no significant difference in recurrence rates between radiotherapy and excision in cases of melanoma in situ.[121] In some cases where surgery is not an option, such as uveal or mucosal melanoma, radiation may be considered as a primary therapeutic option.[122] For cases of inoperable mucosal melanoma of the head and neck region, retrospective studies revealed 5-year survival rates between 15.4% and 28.3% for patients receiving radiation therapy alone.[123] In uveal melanoma, episcleral brachytherapy is the most common approach for preserving eye function. Brachytherapy involves placing radioactive seeds in the eye next to the tumor to deliver a high dose of radiation while preserving the normal tissue.[124] A study comparing outcomes following brachytherapy with Iodine-125 or enucleation (removal of the eye) found no significant difference in mortality between the two cohorts.[103]

In some cases of cutaneous melanoma, it may be difficult to obtain wide negative margins, so adjuvant radiation therapy may be considered. Radiation therapy is sometimes used following lymph node dissection, especially if multiple lymph nodes contain cancer cells. This is done to prevent the further spread of disease. In a Phase III study, 250 patients with positive lymph nodes were randomized to receive adjuvant radiation or observation.[125] After 5 years of follow up, the patients receiving radiation were less likely to relapse, but there was no significant difference in survival, and patients receiving radiation therapy were more likely to experience lymphedema.[126] Adjuvant radiation can also reduce the risk of local recurrence following head and neck mucosal melanoma following complete surgical resection.[122]

Stereotactic radiosurgery (SRS) is a minimally invasive technique that delivers focused, externally generated ionizing radiation without the need to make incisions. For patients with brain metastases, SRS can deliver a high dose of targeted radiation while preserving normal brain tissue. There are three types of SRS technology used to deliver radiation to the brain. Linear accelerators, also known as CyberKnife and TrueBeam, use X-rays to target malignancies in a single session, or up to five for larger tumors. Linear accelerators utilize electron beams for superficial targets or radiographic beams for deeper internal tumors. GammaKnife uses small beams of gamma rays, primarily to treat large to medium tumors. Proton beam therapy is the newest form of SRS and can target tumors in a single session.[122] For multiple brain metastases, SRS is preferred over whole brain radiation therapy, as SRS reduces the risk of neurocognitive toxicities.[127]

Radiation therapy can induce the abscopal effect, whereby targeting a tumor at one site can lead to regression of metastases at a distant site. Although this effect is not fully understood, studies have demonstrated that irradiated tumors produce tumor associated antigens (TAA) during cellular damage. TAAs can stimulate a CD8+ T cell, which promotes an anti-tumor immune response. Although the abscopal effect is rare, it is hypothesized that combining radiation therapy with immunotherapy can "boost" the abscopal effect, leading to regression of metastatic disease. Further understanding of the abscopal effect is greatly needed, including predictive biomarkers. Recruitment for clinical trials studying the synergistic effect of simultaneous radiation and immunotherapy treatments are ongoing. These trials may be able to provide more insight into which patients may benefit from combination radiation therapy and immunotherapy.

Table VIII: Chemotherapy for Melanoma

Drug Name *(Brand Name)*	**Year Approved**	**Description of Use**	**Status**
Dacarbazine, Dimethyl Triazeno Imidazol Carboxamide, Imidazole Carboxamide, DIC, DTIC, *DTIC-Dome*	1975	An alkylating agent, this chemotherapeutic damages DNA, which prevents cell division. Used in patients with advanced or metastatic melanoma.	Perhaps in combination with other approved therapeutics.
Recombinant Interferon Alfa-2b *(Intron® A)*	1995	An adjuvant immunotherapy used for adults with high-risk melanoma to delay recurrence by stimulating the patient's immune system to attach melanoma cells. Generally used in combination with surgical approaches.	As an adjuvant therapy.
Aldesleukin, Interleukin-2 (*PROLEUKIN®)*	1998	Systemic immunotherapy that uses recombinant IL-2 to promote the patient's immune system to target melanoma cells. Used to treat metastatic melanoma.	As an adjuvant therapy and experimentally in combination with adoptive T cell transfer therapy.
Recombinant Peginterferon Alfa-2b PEG-Intron *(Sylatron™)*	2011	An adjuvant immunotherapy used for adults with high-risk melanoma to delay recurrence by stimulating the patient's immune system to attach melanoma cells. Generally used in combination with surgical approaches.	As an adjuvant therapy.
Ipilimumab *(Yervoy)*	2011	A checkpoint inhibitor that inhibits CTLA-4, which in turns boosts the immune system and promotes T cells to attack cancer cells. Used as a systemic immunotherapy to control progression and relive symptoms. Also used as an adjuvant therapy for Stage III patients to reduce risk of recurrence after surgery.	Currently in use as a monotherapy or in combination with nivolumab (2014).
Vemurafenib *(Zelboraf®)*	2011	Signal transduction therapy that inhibits the activity of a BRAF mutation (V600E) which, if left unhindered, promotes abnormal cell growth and division. Administered as a systemic targeted	Currently in use as combination therapy with cobimetinib (2015) and/or the PD-L1 inhibitor atezolizumab (2020).

Drug Name *(Brand Name)*	Year Approved	Description of Use	Status
		therapy alone or in combination with Cobimetinib to slow or stop disease progression.	
Dabrafenib Mesylate *(Tafinlar®)*	2013	Signal transduction therapy that inhibits the activity of a BRAF mutation (V600E) which, if left unhindered, promotes abnormal cell growth and division.	Administered as a systemic targeted therapy alone or in combination with Trametinib to slow or stop disease progression.
Trametinib *(Mekinist®)*	2013	Signal transduction therapy that inhibits the activity of MEK in patients with BRAF mutation (V600E, V600K) which, if left unhindered, promotes abnormal cell growth and division.	Administered as a systemic targeted therapy alone or in combination with Dabrafenib (2014) to slow or stop disease progression.
Pembrolizumab *(KEYTRUDA)*	2014	A humanized monoclonal antibody that inhibits PD-1, which promotes the immune system's ability to fight melanoma.	Used in patients whose cancer cannot be surgically removed or has metastasized and patients who have had surgery to remove cancer that has spread to lymph nodes.
Cobimetinib *(COTELLIC)*	2015	Signal transduction therapy that inhibits the activity of MEK in patients with BRAF mutations (V600E, V600K) which, if left unhindered, promote abnormal cell growth and division.	Administered as a combination systemic therapy with Vemurafenib (2015) or Vemurafenib + Atezolizumab (2020) to slow or stop disease progression.
Nivolumab *(OPDIVO)*	2014	A humanized monoclonal antibody that inhibits PD-1, which promotes the immune system's ability to fight melanoma.	Used in patients whose cancer cannot be surgically removed or has metastasized, sometimes with ipilimumab (2015), and patients who have had surgery to remove cancer that has spread to lymph nodes.
Talimogene Laherparepvec *(IMYLGIC)*	2015	Genetically modified live oncolytic virus that replicates within cancer cells and produces an immunostimulatory protein. Used in melanoma in skin and lymph nodes that cannot be surgically removed. Local treatment in patients whose disease has recurred after surgery.	Optimal for patients with unresectable but injectable tumors. Used as first- or second-line therapy.
Binimetinib *(Mektovi®)*	2018	Signal transduction therapy that inhibits the activity of MEK in patients with BRAF mutations (V600E, V600K) which, if left unhindered, promote abnormal cell growth and division.	Administered as a combination systemic therapy with Encorabenib to slow or stop disease progression.

Drug Name *(Brand Name)*	Year Approved	Description of Use	Status
Encorafenib *(Braftovi™)*	2018	Signal transduction therapy that inhibits the activity of a BRAF mutation (V600E) which, if left unhindered, promotes abnormal cell growth and division.	Administered as a combination systemic targeted therapy with Binimetinib to slow or stop disease progression.
Atezolizumab *(Tecentriq)*	2020	Humanized monoclonal antibody that inhibits PD-L1, expressed on tumor cells. Blocks engagement of PD-L1 with PD-1 expressed on T cells.	Administered as a systemic combination therapy with Vemurafenib and Cobimetinib.

Chemotherapy

Prior to the development of immunotherapy and targeted therapy, chemotherapy was the only systemic treatment option for metastatic melanoma patients (Table VIII). Today, other therapies such as checkpoint inhibitors and targeted therapeutics are considered first line drugs for patients with unresectable or metastatic disease. Dacarbazine (DTIC) was approved by the FDA in 1975 and is an alkylating agent that works by methylating the guanine nucleotide in DNA, preventing the formation of the double helix (Table VIII). This causes the DNA strands to break, thus affecting the ability of the cells to multiply. As a result, dacarbazine is most effective on cells that are rapidly dividing, such as cancer cells, but also hematopoietic cells and cells in the gastrointestinal tract.[128] DTIC is a pro-drug that is converted in the liver to its active compound, 5-[3-methyl-triazen-1-yl]-imidazole-4-carboxamide (MTIC). An analysis of five trials concluded the average one-year overall survival (OS) for DTIC monotherapy was 27%.[1] Another large meta-analysis study demonstrated that only 15% of metastatic melanoma patients responded to DTIC, and studies have not shown any survival benefit for patients receiving DTIC.[129]

Temozolomide (TMZ) is also a pro-drug and an analog of DTIC; however, TMZ does not require the liver for conversion to MTIC. TMZ also crosses the blood-brain barrier but does not appear to have any effect on reducing brain metastases. As an oral agent, TMZ has the ability to be administered on an extended dosing schedule, allowing for 6 weeks of continuous drug exposure compared to the standard schedule for 5 days per month. However, a Phase II trial using extended dosing TMZ demonstrated only a 12.5% response rate, which is not an improvement over DTIC or standard dosing TMZ.[130]

Nitrosureas and platinum-based drugs, which are also alkylating agents, have been used to treat metastatic melanoma as single agents. In a Phase III clinical trial comparing the nitrosurea fotemustine to DTIC, fotemustine demonstrated an overall response of 15.2% versus the 6.8% response rate of DTIC. Fotemustine prevented median time to brain metastasis, 22.7 months compared to 7.2 months for DTIC.[131] As a single agent, cisplatin has an average response rate of 14.4%. In clinical trials, carboplatin was shown to have a response rate similar to cisplatin. The taxanes, docetaxel and paclitaxel, which work by disrupting microtubule function, which is important in dividing cells, have also been used to treat melanoma. In Phase II trials, docetaxel demonstrated an average response rate of 11.4%. Multiple Phase I/II trials with different dosing schedules have been carried out using paclitaxel with partial responses of 12%-15.6%.[128]

In cases where there are multiple, large metastases in the extremities, surgical excision may not be possible. For these patients, isolated limb perfusion (ILP) or infusion (ILI) with chemotherapeutic agents melphalan and actinomycin D may be considered.[109] This is a surgical procedure in which blood flow of the limb is separated from the rest of the body and a high dose of chemotherapy is circulated through the limb.

Melphalan is an alkylating agent, and actinomycin D inhibits transcription. ILI is considered less invasive, but both are effective at treated cases of locally advanced disease (81%-90% response after ILP and 41%-53% response after ILI). In a study of 26 patients with advanced melanoma, the combination of ILI with melphalan and ipilimumab resulted in 58% PFS at 1 year, suggesting that the combination of localized chemotherapy and checkpoint blockade could produce a durable therapeutic response.[132]

Several combination therapies have also been tested in clinical trials. The Dartmouth Regimen (DTIC/carmustine/cisplatin/tamoxifen) was first described in 1984 and demonstrated a 55% response rate in 20 melanoma patients.[133] However, a Phase III trial compared the Dartmouth Regimen to single agent DTIC failed to replicate these results in 240 patients with metastatic melanoma; the response rate of the combination treatment was 18.5% compared to 10.2% response in the DTIC cohort.[134] This difference was not statistically significant, nor was there a significant difference in survival. The MD Anderson Cancer Center developed the combination of cisplatin/vinblastine/DTIC (CVD) that demonstrated a response rate of 40% with a 1-year survival of 50%. A Phase II trial combining cisplatin and paclitaxel demonstrated a 20% response rate with a median survival of 9 months.[128]

Uveal melanoma is highly resistant to systemic chemotherapies. However, for cases where metastasis to the liver has occurred, liver-directed therapy may be an option. In a Phase III trial, patients with uveal metastatic melanoma to the liver were treated with fotemustine delivered via hepatic arterial infusion or intravenously. Hepatic arterial infusion improved PFS (median 4.5 months versus 3.5 months) but did not result in statistically significant differences in overall survival (median 14.6 months versus 13.8 months). Percutaneous hepatic perfusion (PHP) delivers high doses of chemotherapy to the liver with minimal systemic exposure. In a Phase III trial comparing PHP with melphalan to supportive care, there was a significant difference in progression free survival (245 days versus 49 days) and overall response rate (34.1% versus 2.0%), but no survival benefit.[103]

Data regarding systemic therapy for advanced mucosal melanoma is limited. Studies treating patients with standard chemotherapy regimens demonstrated response rates similar to those seen in cutaneous melanomas.[135] No data regarding the use of conventional chemotherapy to treat acral melanoma was found.

TARGETED THERAPY

BRAF Inhibitors

The clinical implementation of BRAF and MEK inhibitors for treating BRAF-positive metastatic melanoma has significantly increased both OS and progression free survival (PFS).[136] The first BRAF inhibitor, vemurafenib, was approved by the FDA in 2011, followed by dabrafenib in 2013 and encorafenib in 2018. In pre-clinical studies, vemurafenib-inhibited MAPK activation, leading to cell cycle arrest and apoptosis in BRAF V600E melanoma cells. In mouse models, a 14-day dose of vemurafenib delivered orally demonstrated a dose-dependent anti-tumor response without.[137] Likewise, dabrafenib inhibits the MAPK pathway in BRAF V600E cells, leading to tumor regression in xenograft mouse models.[138] As monotherapies, vemurafenib and dabrafenib improved response rates, PFS, and OS in patients with BRAF V600E or V600K mutations, compared to standard chemotherapy (dacarbazine).[139] Encorafenib is a highly selective second-generation BRAF inhibitor with a significantly longer half-life (30 hours) than vemurafenib and dabrafenib (30 minutes and 2 hours, respectively).[140] In clinical trials, encorafenib monotherapy was more effective than vemurafenib monotherapy.[141]

MEK Inhibitors

Mutations in BRAF and NRAS activate the MAPK pathway which increases cellular proliferation. In vitro, MEK inhibitors are more effective at inhibiting BRAF-mutant melanoma cell lines than BRAF inhibitors. Additionally, MEK inhibitors reduce proliferation of BRAF-mutant melanoma xenografts in mouse studies.[142] Trametinib was the first MEK inhibitor to receive FDA approval in 2013. Trametinib is an orally available inhibitor of MEK1 and MEK2, which in turn inhibits the downstream target phosphor-ERK1/2. In the Phase III METRIC trial, BRAF-mutant patients were randomized to trametinib, dacarbazine, or paclitaxel treatment. Trametinib monotherapy demonstrated an improved response rate, median PFS, and 6-month survival rates.[143]

Combination BRAF/MEK Treatment

Resistance to monotherapy is common, with a median PFS of 6-8 months. BRAF inhibitor monotherapy also leads to the development of secondary cutaneous malignancies in 19%-26% of patients; further investigation of this phenomenon discovered that BRAF inhibition in BRAF wild type cells leads to MAPK pathway hyperactivation.[139] Therefore, it is recommended that BRAF inhibitors be used in combination with MEK inhibitors for the treatment of metastatic melanoma. In 2015, the FDA approved the use of the MEK inhibitor cobimetinib in combination with vemurafenib for metastatic melanoma patients who were not candidates for surgery. In the Phase III coBRIM trial, locally advanced or metastatic melanoma patients with the BRAF V600 mutation were randomized to receive vemurafenib and cobimetinib or vemurafenib alone. The median PFS for the combination of vemurafenib and cobimetinib was reported as 9.9 months, compared to 6.2 months for vemurafenib monotherapy. Additionally, the combination therapy reduced the number of secondary cutaneous cancers.[144] In 2018, the FDA approved the combination of dabrafenib and trametinib as an adjuvant treatment for melanoma patients with BRAF V600E or V600K mutations and lymph node involvement, following complete resection of disease. This decision was based on the results of the Phase III COMBI-AD trial, where Stage III melanoma patients were randomized to receive dabrafenib and trametinib, or placebo. Patients receiving the combination therapy demonstrated a statistically significant improvement in relapse free survival (58%), compared to the placebo group (39%). Additionally, the 3-year overall survival rate was 86% for the combination therapy group compared to 77% for the placebo group.[145] Also in 2018, the FDA approved the combination of encorafenib with the MEK inhibitor binimetinib for metastatic melanoma patients with unresectable disease and a mutation in either BRAF V600E or BRAF V600K. This approval was based on the Phase III COLUMBUS trial, where patients were randomized to receive encorafenib plus binimetinib, encorafenib alone, or vemurafenib alone. The median PFS was 14.9 months in the encorafenib and binimetinib group, compared to 9.6 months for encorafenib monotherapy and 7.3 months for vemurafenib monotherapy.[141]

Opportunities and Areas of Further Investigation

Additional targeted therapies are greatly needed for non-BRAF mutated and non-cutaneous melanomas. NRAS mutations are present in approximately 25% of cutaneous melanomas, and although the MAPK pathways is downstream of NRAS, attempts to target these cases with MEK inhibitors have only demonstrated modest response rates. NF1 mutations are present in about 15% of cutaneous melanomas but response to MEK inhibitors is variable. Further characterization of these melanomas are needed to improve targeted treatment.[146]

Mutations and amplifications in KIT have been identified in mucosal and acral melanomas. A Phase II trial investigating the efficacy of tyrosine kinase inhibitor imatinib in patients with KIT mutations or amplifications had a 29% Overall Response Rate (ORR), suggesting imatinib as a promising therapeutic.[147] However, another Phase II trial investigating a related tyrosine kinase inhibitor, dasatinib, for mucosal and acral

melanomas was discontinued due to adverse events and low response rate.[148] Genomic alterations in the telomerase reverse transcriptase gene (TERT) are present in over 40% of acral melanomas. TERT is silenced in most normal cells, making it an attractive therapeutic target for acral melanoma. Pharmacologic strategies to inhibit TERT function may be a promising avenue for treating this rare type of melanoma.[149]

Mutations in GNAQ and GNA11 are present in approximately 85% of uveal melanomas, but these two genes have been difficult to target therapeutically. Clinical trials to target downstream effectors such as MAPK and PI3K/AKT pathways have demonstrated minimal efficacy.[146] Mutations in the tumor suppressor BAP1 are present in 60% of uveal melanoma patients but is also a difficult therapeutic target. A drug screen identified the histone deacetylase inhibitor quisinostat as a potential candidate for BAP1-mutant melanoma.[150]

Immunotherapy

Immunotherapy is a type of therapeutic that works by either activating or suppressing the immune system to treat a disease. In cancer, immunotherapy works by stimulating the immune system to kill cancer cells. For Stages I-III melanoma, adjuvant therapy with an immune checkpoint inhibitor or targeted therapy might be recommended to lower the risk of recurrence. For patients with Stage IV metastatic melanoma, metastasis that cannot be surgically removed may be treated with immunotherapy. Checkpoint inhibitors are usually the first drugs tried for patients with tumors that lack the BRAF mutation.

Cytokine Therapy

Interleukin-2 (IL-2) is a cytokine that stimulates the expansion and survival of T cells. In 1985, 25 patients with metastatic cancer were treated with high-dose IL-2; 4 of the 7 patients with metastatic melanoma exhibited regression of their tumors.[151] The FDA approved the IL-2 in 1998 to treat metastatic melanoma patients. A meta-analysis of results from 270 metastatic melanoma patients entered in eight clinical trials testing high-dose IL-2 reported that the ORR was 16%, with 17 Complete Response (CR) (6%) and 26 Partial Response (PR) (10%).[152] Likewise, a meta-analysis of 243 melanoma patients treated at the University of Pittsburgh revealed an OR of 18.1% and a CR of 8%.[153] Administration of IL-2 is given intravenously in a hospital setting due to the side effects, including fever, chills, aches, drowsiness, decreased blood cell counts, and swelling.[151]

Interferon (IFN) is another cytokine that stimulates expansion of T cells. In 1995, interferon became the first adjuvant therapy approved by the FDA to treat melanoma following surgical removal of tumors. A randomized, three-arm trial evaluated the efficacy of high-dose IFNα2b (HDI) for 1 year, low-dose IFNα2b (LDI) for 2 years, or observation in Stage IIB and III melanoma patients. The 5-year RFS rate was 44% for HDI, 40% for LDI, and 35% for observation only; however, there was no benefit in OS.[154] In 2011, the FDA approved pegylated IFN (PEG-IFN) for the adjuvant treatment of melanoma patients following surgical resection and lymphadenectomy. The conjugation of recombinant IFNα2b to polyethylene glycol protects IFNα2b from degradation and increases the biological half-life. Patients were randomized to either PEG-IFN or observation for 5 years following surgical resection of their melanoma tumors. PEG-IFN treatment resulted in a statistically significant median RFS of 34.8 months, compared to 25.5 months in the observation only group, with no significant difference in OS.[155]

Immune Checkpoint Inhibitors

The most significant advance in the treatment of melanoma came with the FDA approval of immune checkpoint inhibitors. T cells can express several checkpoint molecules that function to “fine-tune” or

regulate hyperactivation of the immune response. Cytotoxic T lymphocyte antigen 4 (CTLA-4) is strongly induced following T cell activation. The biological role of CTLA-4 is to antagonize activating signaling pathways in the T cell. Tumor cells can engage CTLA-4 as a way to evade the anti-tumor immune response. The discovery of CTLA-4 as a negative regulator of T cell activation lead to the hypothesis that CTLA-4 could be blocked therapeutically in order to reactivate anti-tumor T cells. In pre-clinical studies, anti-CTLA-4 antibodies blocked binding of CTLA-4 to its ligands CD80 and CD86, enhanced anti-tumor immunity and lead to tumor regression.[156] Ipilimumab, an anti-CTLA-4 monoclonal antibody (mAb) was approved by the FDA for non-resectable Stage III or IV melanoma. In the pivotal Phase III trial, 676 patients were randomized to receive either ipilimumab, antigenic gp100 peptide vaccine, or both. The median OS in the ipilimumab + gp100 group was 10 months compared to 10.1 months for ipilimumab alone and 6.4 months for gp100 alone. After 12 weeks of follow up, the rates of progression-free survival were 49.1% with ipilimumab+gp100, 57.7% with ipilimumab alone, and 48.5% with gp100 alone.[157] As gp100 did not offer any additional benefit, the FDA chose to approve the use of ipilimumab alone. A second Phase III trial investigated ipilimumab in combination with dacarbazine for metastatic melanoma patients with previously untreated disease. After 3 years, the 3-year OS was estimated at 20.8% for ipilimumab + dacarbazine compared to 12.2% for dacarbazine + placebo.[158]

Programmed cell death 1 (PD-1) is another immune checkpoint molecule expressed on T cells. PD-1 is upregulated on T cells after stimulation of the T cell receptor and binds to ligands PD-L1 and PD-L2 to negatively regulate T cell activation. PD-1 signaling is critical for controlling activation and proliferation of differentiated effector T cells; when PD-1 engages its ligands, it can induce T cell exhaustion, a progressive loss of effector functions. Tumor cells can exploit this mechanism by upregulating PD-L1 or PD-L2 to induce T cell exhaustion and promote a tumor microenvironment that enables tumor growth.[159] In pre-clinical studies, cancer cell lines found to overexpress PD-L1 or PD-L2 blocked CD8+ T cell function; in mice, these cell lines demonstrated increased tumor burden and metastasis. Inhibiting PD-1 with neutralizing antibodies reversed these effects and enhanced cytotoxic CD8+ T cell responses.[160] In 2014, the FDA approved pembrolizumab, a monoclonal antibody that blocks PD-1 from binding to PD-L1 and PD-L2. Accelerated approval for pembrolizumab was based on the Phase Ib KEYNOTE-001 trial, in which untreated or previously treated (with ipilimumab or BRAF inhibitors) unresectable metastatic melanoma patients received either 2 mg/kg or 10 mg/kg ipilimumab. The 5-year analysis of this trial revealed that the OS was 34% in all patients with a median OS of 23.8 months. The estimated 5-year PFS rate was 21% with a median PFS of 8.3 months.[161] In 2019, the FDA expanded its approval for pembrolizumab as an adjuvant treatment for patients following complete lymph node resection.

A second PD-1 inhibitor, nivolumab, also received FDA approval in 2014 for the treatment of patients with unresectable or metastatic melanoma who experienced disease progression following ipilimumab or a BRAF inhibitor. This approval was based on the Phase III Checkmate-037 trial in which patients were randomized to receive nivolumab or chemotherapy (dacarbazine or paclitaxel combined with carboplatin). This study reported a 32% ORR for patients receiving nivolumab compared to 10% ORR for patients treated with chemotherapy.[162] Checkmate-066 randomized previously untreated, non-mutated BRAF metastatic melanoma patients to either nivolumab or dacarbazine; at 1 year, the OS was 72.9% for the nivolumab group compared to 42.1% for the dacarbazine group, with a median PFS of 5.1 months for the nivolumab group and 2.2 months for the dacarbazine group.[163] In 2017, the FDA expanded its approval for nivolumab as an adjuvant treatment for patients with lymph node involvement or metastases following complete resection. Both pembrolizumab and nivolumab have demonstrated better PFS and OS compared to ipilimumab.[164,165] It is unknown why anti-PD-1 is more effective than anti-CTLA-4.

Following the success of checkpoint inhibitors as monotherapy, in 2015 the FDA approved nivolumab in combination with ipilimumab based on the initial results of the Checkmate-067 trial. Patients with previously untreated, unresectable, or metastatic melanoma were randomized to receive either nivolumab monotherapy, ipilimumab monotherapy, or a combination. After 5 years of follow-up, the median OS for the combination group was greater than 60 months (median not reached), compared to 36.9 months for nivolumab monotherapy and 19.9 months for ipilimumab monotherapy. The 5-year OS was 52% for the combination group compared to 44% for nivolumab monotherapy and 26% ipilimumab monotherapy.[166]

As checkpoint inhibitors stimulate unrestrained T cell proliferation, immune related adverse events (irAEs) are common. Approximately 80% of patients treated with checkpoint inhibitors report adverse events, including enterocolitis, hepatitis, and dermatitis. Most irAEs can be managed and reversed if diagnosed early; systemic corticosteroid treatment, along with discontinuation of checkpoint inhibitor, is recommended.[167]

Oncolytic Virus Therapy

Talimogene laherparepvec (T-VEC) is an oncolytic virus approved by the FDA in 2015 for intralesional treatment of metastatic melanoma. T-VEC is derived from herpes simplex virus type I, genetically engineered to allow for selective tumor replication and tumor-associated antigen presentation. Infection of tumor cells with T-VEC results in tumor lysis and release of tumor specific antigens, which are processed by dendritic cells and presented to CD8+ T cells. The T cells in turn initiate an anti-tumor response, which causes the release of more tumor specific antigens and amplifies the anti-tumor immune response.[168] In a Phase III trial, 436 patients with unresectable Stage III or IV melanoma were randomized to either intratumoral T-VEC or GM-CSF. The 6-month ORR was 26.4% for T-VEC treatment and 2.1% for GM-CSF treatment. In T-VEC treated patients, regression was seen in both injected and non-injected tumors, and a CR occurred in 10.8% of patients.[169] T-VEC can be implemented as a first- or second-line therapy in patients with unresectable but injectable Stage III or Stage IV disease.[170]

Adoptive T Cell Transfer Therapy

Adoptive T cell transfer therapy (ACT) is a form of personalized medicine that involves isolating tumor infiltrating lymphocytes (TILs) from tumors, expanding them ex vivo with IL-2, and then infusing large numbers of tumor-reactive T cells back into patients along with high-dose IL-2. In the earliest studies of ACT between 1987 and 1992, 86 metastatic melanoma patients were treated with TILs plus IL-2 in two cycles. Fifty-six of these patients were also treated with the immune suppressant cyclophosphamide prior to the first TIL infusion. After 6 weeks, the ORR was 34%.[171] In a follow-up clinical trial, 93 metastatic melanoma patients were given a non-myeloablative lymphodepleting regimen of cyclophosphamide and fludarabine alone or in conjunction with total body irradiation of 2 Gy or 12 Gy prior to TIL infusion. TILs were grown from resected metastatic melanoma lesions in culture with high-dose IL-2. At a median follow up of 62 months, 52 patients had an objective response, with 20 patients experiencing a complete regression.[172] In addition to the ACT studies conducted at the National Institutes of Health, other groups have reported similar ORs, such as Moffitt Cancer Center (38% ORR) and MD Anderson Cancer Center (48% ORR). ACT for metastatic melanoma is not FDA approved, but can be considered on an experimental basis for patients who experience recurrence following checkpoint blockade. One benefit of ACT is that TILs can cross the blood-brain barrier, and regression of brain metastasis has been observed.[173] Currently, a Phase II, multi-center trial sponsored by Iovance Biotherapeutics (NCT02360579) is testing the safety and efficacy of autologous TILs in metastatic melanoma patients. This study is estimated to be completed by 2024.

Several toxicities are associated with ACT. Lymphodepletion with cyclophosphamide and fludarabine can cause infection and bleeding as a result of bone marrow suppression, and in rare cases, lymphoproliferative diseases. High-dose IL-2 can cause capillary leak syndrome.[174] Cytokine release syndrome (CRS) has also been reported in melanoma patients receiving ACT. CRS is a systemic inflammatory disorder caused by a massive release of cytokines by the infused T cells and other immune cells that participate in the anti-tumor immune response. CRS is characterized by fever, tachycardia, and hypotension. Patients experiencing CRS require intensive supportive care, including vasopressors and blood product transfusions. Glucocorticoids are considered the first-line treatment for mild CRS but can decrease the efficacy of ACT. Tociluzimab, an IL-6 receptor antagonist, can ameliorate CRS toxicity without interfering in the ACT anti-tumor immune response.[160,174]

Combination Therapies

In 2020, the FDA approved the PD-L1 inhibitor atezolizumab in combination with cobimetinib and vemurafenib for patients with unresectable or metastatic BRAF V600 mutation positive melanoma. This approval was based on the results of the Phase III IMspire150 trial. All patients enrolled in the trial were treated with cobimetinib and vemurafenib for 28 days, then randomized to receive atezolizumab or placebo in combination with the BRAF/MEK inhibitors. The median PFS was 15.1 months for the atezolizumab group compared to 10.6 months for the placebo group.[175]

Opportunities and Areas for Further Investigation

Although checkpoint inhibitors have revolutionized the treatment of metastatic melanoma, not all patients will respond to anti-PD-1 or anti-CTLA-4. Additionally, immune checkpoint inhibitors are only FDA-approved for the treatment of cutaneous melanomas. Multiple agonistic and antagonist immune checkpoints are currently in clinical trials, including GITR, CD40, VISTA, TIM-3, LAG-3, and TIGIT. Single cell analysis of the uveal melanoma tumor microenvironment revealed that tumor infiltrating CD8+ T cells express LAG-3 rather than PD-1 or CTLA-4.[176] A Phase II clinical trial to study the efficacy of nivolumab and a LAG-3 inhibitor relatimab for metastatic uveal melanoma will initiate soon (NCT04552223). The development of biomarkers to predict which patients will respond to checkpoint inhibition and/or develop irAEs is also an active area of investigation. Another challenge is that non-cutaneous melanomas are rare, and thus, it is difficult to recruit patients for clinical trials. One retrospective analysis of acral melanoma patients suggests that ipilimumab has similar OS to cutaneous cases; this study only analyzed 35 patients so more information is needed before ipilimumab could be considered a viable treatment for acral melanoma.[177]

Another active area of investigation is understanding the role of the tumor microenvironment, and how to transform “cold” tumors into “hot” tumors that will respond to checkpoint inhibition. This would have implications for non-cutaneous forms of melanoma that are traditionally characterized as low mutation burden, low PD-1 expression, and poor immune infiltration in the tumor microenvironment. Some tumors are characterized by a suppressive metabolic microenvironment; multiple therapeutic strategies are under investigation to target the metabolic imbalances in the tumor. For example, combining checkpoint inhibitors with metformin activates the AMPK pathway, which is important for T cell function.[178] Amino acids such as arginine, tryptophan, and glutamine play crucial roles in T cell function; targeting these amino acids and their pathways is a promising avenue for promoting an anti-tumor microenvironment.[179] There is growing evidence that host factors such as the microbiome can impact response to checkpoint inhibition, and currently there are multiple clinical trials investigating modulation of the microbiome to improve response to immunotherapies.[180] Further investigation of the tumor microenvironment may also reveal biomarkers that can help determine additional combinations of targeted therapeutics and immunotherapies.

There has been renewed interest in combining radiation with immunotherapy. There is a need to understand the mechanisms of radiation-induced anti-tumor immunity in order to explore how these two therapies can be combined. Clinical studies are needed to establish optimal radiation doses and scheduling of immunotherapy agents. Predictive biomarkers can inform radiation induced immunological changes, which can identify the most appropriate immunotherapy agent.[181]

Several clinical trials are in progress investigating new oncolytic viruses for melanoma. CAVATAK, an oncolytic strain of Coxsakievirus A21, infects ICAM-1 expressing tumor cells. In a Phase II study (NCT01227551), treatment with CAVATAK resulted in increased CD8+ TILs and PD-L1+ cells. CAVATAK has also been explored in combination with ipilimumab (NCT02307149) and pembrolizumab (NCT02565992). Another clinical study has been initiated to investigate the use of the oncolytic adenovirus ONCOS-102 in combination with pembrolizumab (NCT03003676). Oncolytic virus therapy can be an avenue to create a "hot" tumor microenvironment, which will improve the efficacy of checkpoint inhibitors; this would be beneficial for subtypes of melanoma that have a small number of tumor associated antigens and are classified as "cold" tumors.[182] Oncolytic viruses that can cross the blood-brain barrier, such as oncolytic herpes simplex virus, have potential for targeting brain metastases.[183]

ACT is a promising therapeutic avenue for metastatic melanoma but is not yet FDA approved. ACT is highly personalized, and thus will be very expensive. Chimeric antigen receptor (CAR)-T cell therapy is a variation of ACT in which the patient's T cells are genetically engineered to express receptors that will specifically recognize and target tumor cells. Although CAR-T is approved for several hematological malignancies, it has not demonstrated much efficacy in solid tumors.[184] Treatment with CAR-T can also induce CRS. Biomarkers to predict CRS in patients receiving ACT or CAR-T are also needed. Clinical investigations to study ACT in combination with checkpoint inhibitors or targeted therapies should also be pursued.

SECTION VII: Dormancy, Recurrence, and Metastatic Disease

Dormancy and Metastasis

Metastasis, the spread, establishment, and proliferation of cancer cells in new locations throughout the body, has been classically described in melanoma as the progression from a nevus within the epidermis to melanoma in situ, invasive malignant melanoma, and finally metastatic melanoma.[1] However, increasing evidence suggests that melanoma progression does not fit such a linear model of metastasis and that malignant melanoma exhibits a predilection for early and rapid spread through the lymphatic and circulatory systems.[16,185]

With the initiation of metastasis, cutaneous melanoma cells within the primary tumor may undergo an epithelial-mesenchymal (EMT) transition that enables dissemination.[16] The EMT transition involves the downregulation of adhesion molecules, enabling melanoma cells to detach from the primary tumor. However, this theory is largely based on in vitro studies and while the model provides a potential explanation for initial dissemination, the EMT transition and downregulation of adhesion molecules does not explain how the cells then establish themselves at distal sites.[16,186]

Tumor cells are known to be very sensitive to microenvironmental changes. The stromal microenvironment is a complex milieu of immune cells, extracellular matrix, fibroblasts, keratinocytes, and endothelial cells.[187] Secreted factors can modulate signaling, resulting in either cell death, metastasis, or dormancy. The presence or absence of secreted factors, different cell types, and hypoxia can affect survival within a

foreign microenvironment, growth, and proliferation.[16] Melanoma tumor heterogeneity plays a significant role in metastasis, dormancy, recurrence, and treatment. One hypothesis suggests the tumor microenvironment exerts selective pressure on melanoma cells, resulting in heterogeneity despite clonal expansion, and eventually a subset of the tumor population gains the ability to migrate. Once free of the primary tumor, cutaneous melanoma cells may enter local lymphatic vessels, which enable transit into the sentinel lymph node and entry into systemic circulation via the thoracic duct. Non-canonical Wnt5A, along with β-catenin, has been implicated in melanoma cells converting to an invasive phenotype,[187] with studies in human melanoma lines and mouse models showing conflicting results. Cancer stem cells, miRNAs, and down regulation of metastasis suppressing factors such as KISS1, GPR56, BRMS1, and NEDD9, have also been implicated in initiation, but the exact mechanism by which they contribute remains unknown.

Growing evidence supports the concept of parallel progression. Werner-Klein et al.[188] found that dissemination occurs very early in melanoma, requiring disseminated cells to acquire genetic alterations outside of the primary tumor. Mutations in TERT, a subunit of telomerase, have been observed in up to 43% of cutaneous melanomas. While both BRAF and NRAS mutations have been suggested to have a role in the initiation of metastasis, Werner-Klein et al.[188] found that BRAF mutations were more frequently observed in primary tumor cells (34%) compared to disseminated cells (15%) and that circulating tumor cells with and without BRAF and NRAS mutations were able to establish metastases. However, they also found that mutations in BRAF and CDK2NA emerged when disseminated cells reached a population threshold sufficient to initiate a morphological colony.

Evidence increasingly supports the hypothesis that melanoma dissemination takes place early in disease progression, possibly even before appearance of a primary tumor. It has been observed that 4%-12% of malignant cutaneous melanoma patients show no evidence of a primary tumor.[16,189] The theory of premalignant dissemination proposes that premalignant cells enter circulation before acquiring malignant potential. Premalignant cells, such as benign melanocytic nevi, are often found in lymph nodes (0.33%-7.3% of lymph nodes from non-melanoma patients).[16] Similarly, circulating tumor cells can be observed in uveal melanoma patients up to several years before clinically advanced disease becomes apparent.

Melanoma frequently metastasizes to the lungs, liver, brain, and bone.[185] This organ specificity (organotropism) may be explained by structural and molecular differences in different organs. One theory suggests the presence of fenestrated (having perforations) capillaries make extravasation easier. Based on this, metastatic spread should be most common in the lymph nodes, liver, spleen, and bone marrow and rare in tissues with tight junctions such as the lung and brain. This does not accurately represent the pattern of metastasis clinically observed; especially given that lung and brain are among the most common sites of melanoma metastasis.[16] Cell surface receptors and chemokines, known mediators of cell migration and survival, have also been proposed to explain melanoma organotropism.

The lung is the most common site in regards to distal spread (14%-18%).[185,190] In cutaneous melanoma, the lungs are frequently the first site of metastasis. Acral and, although infrequently, uveal melanomas are also capable of establishing metastases in the lung.[191-193] In cutaneous melanoma, lung-endothelial cell adhesion molecule-1 (Lu-ECAM-1)/CCL2A, integrins, and CXCR4/CCR12 have been implicated in lung metastasis. CXCR4 ligand/CCR12 interactions in particular mediate specificity of adhesion and lead to activation of mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinases (ERK) and phosphoinositide 3-kinase (PI3K) anti-apoptotic pathways.[16] Depletion of CD8+ T cells accelerated onset of lung metastases in a RET.AAD mouse model of melanoma, suggesting that CD8+ T cells play a role in tumor cell growth kinetics.[189]

Cutaneous melanoma commonly results in brain metastases, with roughly 60% of metastatic melanoma patients eventually developing brain metastases.[194] Acral and mucosal melanomas can also metastasize to the brain, but uveal melanoma does not. The brain presents a unique and tightly regulated microenvironment. Melanoma cells must first pass through the blood-brain barrier, and there is no lymphatic system to ease transport.[16] Superficial spreading melanoma is particularly adept at gaining access to the brain. Numerous factors have been implicated in brain metastasis (Table IX) pathway, which has been investigated for its role in establishing brain metastases. However, little investigation has been done to evaluate PI3K-AKT pathway inhibitors ability to prevent brain metastases.

Melanoma patients exhibit a high incidence of leptomeningeal disease (LMD). Typically associated with late stage disease and concurrent central nervous system (CNS) metastases, LMD is the invasion of the acellular space containing cerebral spinal fluid, by cancer cells. There are currently no available models and the molecular mechanisms behind LMD are unknown, although work done in breast and lung cancer suggests the increased expression of innate immunity mediator complement 3 (C3) in cells that metastasize to the leptomeningeal space may be involved. Entry perineurally along the cranial nerves and spinal roots in particular is linked to desmoplastic melanoma, although this route is rarely observed in other subtypes.[195]

Table IX: Factors influencing brain metastases); including the phosphor-inositide-3-kinase/AKT (PI3K-AKT)

Factors Influencing Brain Metastases	***Summary of Involvement***
Phosphor-inositide-3-kinase/AKT (PI3K-AKT) pathway	Migration and invasion, mediates response to BRAF and MEK inhibitors[194]
Cyclin-dependent kinase inhibitor 2A (CDKN2A)/ cyclin-dependent kinase 4 (CDK4) axis	Mediate responsiveness to CDK4 inhibitors[194]
Autophagy	Exact involvement is unknown[194]
TGF-β signaling	May promote invasion [16]
Transferrin receptor	Mediate human melanoma cell lines localization to brain in mouse models[16]
Neurotrophins and neurotrophin receptors	Potentially help promote colonization of brain by regulating production of ECM-degradative enzymes (heparanase)[16]
p75/ tropomyosin receptor kinase C (TrkC)	Interact with nerve growth factor (NGF) and neurotrophin-3 (NT-3) to mediate metastasis[16]

While liver metastasis is only observed in 10%-20% of cutaneous melanoma patients[16] and occurs late in progression, the liver is the first site of metastasis in 80%-90% of uveal melanoma patients.[193] Laminin-1, an extracellular matrix glycoprotein, has been shown to mediate liver metastasis in a cutaneous melanoma model. Laminin-1, in conjunction with vascular cell adhesion protein 1 (VCAM-1), is thought to interact with integrins in order to mediate adhesion specificity and survival.[16] Uveal melanoma exhibits a unique metastatic pattern among the various melanoma subtypes, and the mechanism by which uveal melanoma establishes liver metastases remains largely speculative.[193]

Melanoma metastasis to bone and the gastrointestinal tract typically occurs during Stage IV cancer progression.[16] Melanoma cells are able to colonize the bone, typically the axial skeleton, and undergo

osteolytic metastasis.[189] Bone metastases are observed in 11%-17% of Stage IV cutaneous melanoma patients. Though the mechanism is unknown, there is a subset of patients whose melanoma actually metastasizes to the bone first. Xenograft studies implicated TGFβR1 as a mediator of bone metastasis in melanoma and inhibition of TGFβR1 blocks melanoma cells from undergoing osteolytic metastasis[16, 189]. Acral, mucosal, and uveal melanomas are also able to metastasize in bone.[191,193,196] Metastasis of the gastrointestinal tract preferentially inhabit the small intestine.[16] CCL25 and CCR9 have been proposed as mediators of intestinal metastases. Roughly 86% of melanoma metastases in the small intestine express CCR9, which may interact with CCL25 in the microenvironment.

Metastasis may not occur immediately, as suggested by the extended disease-free periods experienced by melanoma patients before their first recurrence. The period between removal of the primary tumor or disappearance of the primary lesion and the first recurrence is known as dormancy. During this time, minimal residual disease and micrometastases remain asymptomatic.[16,189] Dormancy in melanoma has been observed to last months to years, and in a subset of cutaneous melanoma, ultra-long dormancy results in recurrence decades later.[16]

Restriction of vascular supply is recognized as an inducer of dormancy (angiogenic dormancy). Angiogenesis is regulated by stromal cells such as innate and adaptive immune cells and fibroblasts.[189] Biopsies of human melanoma tissue shows that macrometastases have twice the density of microvessels as micrometastases. Additionally, single disseminated melanoma cells have been shown to establish themselves throughout the lung, but only the cells adjacent to vasculature or along the lung surface initiate growth.[16] Cells subject to angiogenic dormancy require a shift in the balance between pro- and anti-angiogenic factors to metastasize. The upregulation of thrombospondin-1 (TSP-1), a secreted glycoprotein with anti-angiogenic factors and a potential oncogenic role, is associated with dormant phenotypes, but the mechanism in melanoma is unknown.[189] Kienast et al.[189] observed that melanoma cells in the brain used preexisting vasculature (vessel co-option) to disseminate and grow. In other cancers, endothelial cells have been shown to regulate dormancy by creating a dormant niche around stable microvasculature or a metastatic niche via secreted factors, but this has not yet been shown in melanoma.

Numerous signaling pathways have been implicated in cellular dormancy in melanoma (Table X). For example, downregulation of mammalian target-of-rapamycin (mTOR) is common in dormant cells. mTOR regulation has been shown to be important in dormancy induction and survival of dormant cells exposed to metabolic stress conditions.[189] The AMP-activated serine/threonine protein kinase (AMPK) pathway, which, in conjunction with human liver kinase B1 (LKB1), activates mTOR, which results in the downregulation of growth stimulating pathways and the coordination of survival signaling through mechanisms such as autophagy in response to metabolic stress conditions in the microenvironment (i.e., nutrient deprivation, hypoxia).[189] Serine/threonine kinase AKT activation of mTOR results in escape from oncogene-driven senescence. Understanding of how stress pathways modulate cellular growth, apoptosis, and metabolic needs is required. . BRAF inactivates LKB1/AMPK signaling in 10% of melanomas, suggesting an alternative role for the pathway in BRAF-driven melanoma. The relevance of AMPK signaling to dormancy remains unclear. There is a critical need to elucidate the duality of these different pathways, in particular the Wnt signaling pathway, in melanoma.

The cytokines, interferon γ (IFNγ) and interleukin-2 (IL-2), have been proposed as mediators of escape from immunogenic dormancy in melanoma.[189] Appearance of metastatic melanoma in immunosuppressed transplant recipients also strongly supports the theory that immune regulation can induce dormancy in melanoma.[1]

TABLE X: Cellular Factors Influencing Dormancy in Melanoma

Cellular Factors Influencing Dormancy	*Summary of Involvement in Melanoma*
Wnt Signaling and β-catenin	Canonical and non-canonical Wnt signaling play critical role in progression; Wnt5a and Wnt3A[197]
p53-p21 and p16INK4a-RB Pathways	Contribute to melanoma calls bypassing BRAF-induced senescence[37]
Microphthalmia-Associated Transcription Factor (MITF) Signaling	High and low MITF expression in melanoma cells is associated with proliferative and invasive phenotypes, respectively[189]
Indolamine 2,3-dioxygenase/aryl hydrocarbon receptor/27-dependent (IDO1/AhR/p27-dependent) pathway	IFN-β treatment induces melanoma tumor repopulation cells into dormancy through an IDO1/AhR/p27-dependent pathway; disruption of the pathway results in IFN-β mediated apoptosis[198]
mTOR Activation via LKB1/AMPK	Mediate downregulation of growth signaling pathways and upregulation of survival signaling pathways (autophagy) in response to microenvironmental stress[189]
mTOR Activation via PI3K/AKT	Escape from oncogene-driven senescence[189]
RAS/RAF/MEK/ERK Signaling	Exact involvement is unknown, although ERK and p38 have been implicated[189] in dormancy
AXL Receptor Tyrosine Kinase Signaling	Upregulation is coupled with MITF plasticity[189]
Transforming Growth Factor beta (TGF-β) Signaling	Regulates stem cell maintenance; TGFBR3 associated with immune evasion of primary tumors in melanoma[189]

Some of these cellular factors, such as MITF and TGF-β, contribute to dormancy through melanoma plasticity, the ability to shift between an invasive, dedifferentiated, stem-like phenotype and a proliferative phenotype (phenotype switching).[189,198] The degree of MITF expression is associated with different melanoma cell phenotypes. High MITF expression is observed on cells with a proliferative phenotype and low MITF expression on cells with an invasive phenotype. Both low and high MITF expression is detectable in primary tumors, but expression is absent in circulating tumor cells, suggesting that MITF expression may be dependent on microenvironmental factors.[189] Understanding plasticity is critical to understanding the induction of dormancy. How these cells remain dormant while acquiring additional epigenetic modifications, such that the eventual metastases exhibit heterogeneity, over time remains unknown, but the tumor environment has been recognized as a key modulator.[189] Hypoxia is another microenvironmental factor that contributes to cellular dormancy through induction of pathways such as TANK-binding kinase 1 (TBK1) and unfolded protein response (UPR), activation of Wnt signaling, and modulation of phenotype switching.[189]

Physical and biochemical properties of the extracellular matrix (ECM) modulate dormancy. Tissue stiffness has been associated with a proliferative role in melanoma. Studies have shown the attachment of melanoma cells to fibrillary collagen mediates cell cycle arrest and induces dormancy in vitro.[189] The microenvironment, including the ECM, changes with age. Accumulation of cells exhibiting a senescence-associated secretory phenotype (SASP) is associated with widespread changes in epigenetic gene expression and increases in secretion of pro-inflammatory cytokines, chemokines, growth factors, and

proteases.[37] SASP-associated soluble factors are associated with tumor invasion (MMPs, PAIs, tPA, IGFBP, CSF, VEGF) and immune evasion (CXCL1, CXCL2, IL-6, IL-10, GM-CSF).[37] Wnt inhibitor secreted frizzled-related protein 2 (sFRP2) secreted from aged dermal fibroblasts triggers a signaling cascade starting with β-catenin, which results in accumulation of oxidative stress and cellular damage, leading to genomic instability.[187] The potential for antioxidant therapies that could prevent progression is unclear due to the conflicting evidence that, while antioxidants are effective at reducing ROS-mediated DNA damage in primary tumors, the number and burden of lymph node metastases increase in young mice.[187]

Other secreted factors include hormonal influences and metabolic factors. Melanomas diagnosed during pregnancy are often more aggressive, possibly due to the aberrant expression of oestrogen receptors.[37] Studies in other cancers show the metabolic signatures of metastases in the central nervous system can be very distinct from the primary tumor and extracranial metastases, but this has yet to be shown in melanom194.[1]

Escape from dormancy may result in recurrence. A retrospective study using patient data from the Kaiser Permanente Colorado (KPCO) Tumor Registry from 2000-2015 found that overall recurrence of melanoma was observed in 8.8% of patients, compared to previous studies that observed overall recurrence at between 12%-30%.[190,199] Similarly, a prospective study done in Queensland, Australia followed 700 patients diagnosed with high-risk primary melanomas for 2 years post-intervention between 2010-2014. Within 2 years, melanoma recurred in 13.4% of patients.[190] However, only a limited number of studies have focused on melanoma recurrence and no population-based long term studies have been done in the United States. While it's clear that a significant portion of melanoma survivors experience recurrence, the exact mechanism behind recurrence remains unclear.

The anatomical location of the primary tumor appears to play a confounding role in melanoma recurrence. Primary melanomas on the head, neck, and trunk are more likely to recur than melanomas located elsewhere on the body.[16,190] Regional recurrence for cutaneous and acral melanoma typically involve nearby skin, subcutaneous tissue, and regional lymph nodes.[16,191] Depending on the distance from the primary lesion, skin metastases may be categorized as satellite lesions or in transit melanoma.[16] Melanoma can also result in distal skin metastases. Regional lymph nodes are the most commonly affected site overall for cutaneous melanoma (38%).[190] Because the lymphatic endothelium is made up of a poorly defined basement membrane and contains frequent interendothelium gaps, the lymphatic system provides tumor cells easy access into the circulation.[16] Tumor cell invasion of lymph nodes is associated with local immunosuppression. The immunosuppression, in combination with chemokines secreted by endothelial cells lining the lymphatic channels, such as CCL21, may mediate metastasis via the CCR7 receptor on melanoma cells. CXCL21 and CXCR3 have also been implicated in cutaneous melanoma progression through lymph nodes.[16,200] Because of its unique anatomical location, uveal melanoma does not have access to lymph nodes and instead dissemination must take place through local extension or the circulatory system. Local recurrence for uveal melanoma is typically still within the eye.

Melanoma progression remains a significant challenge. A better understanding of the pathogenesis behind the initiation and modulation of metastasis and dormancy is critical to finding new methods to prevent or halt progression and improving patient outcomes. In response to these critical gaps, the MRP encourages research to elucidate the role of the tumor microenvironment, the significance of minimal residual disease, and the identification of new routes of therapeutic prevention to interrupt progression or prevent recurrence.

SECTION VIII: Research Funding
Funding Landscape

Melanoma Research Program

The MRP is focused on investing in research that addresses gaps in prevention, detection, diagnosis, and treatment of melanoma for the benefit of Service members, Veterans, their families, and the American public. In an effort to shift the approach of melanoma prevention, treatment, and long-term care, the MRP has challenged the research community to redefine the concept of prevention to include the entire melanomagenesis process (see Section IV: Prevention).

During its inaugural year, the MRP funded 19 awards, representing 16 unique projects through 4 award mechanisms: Idea Award, Team Science Award, Translational Research Award, and Concept Award (Figure 2), which cover a multitude of scientific research areas (Figure 3).

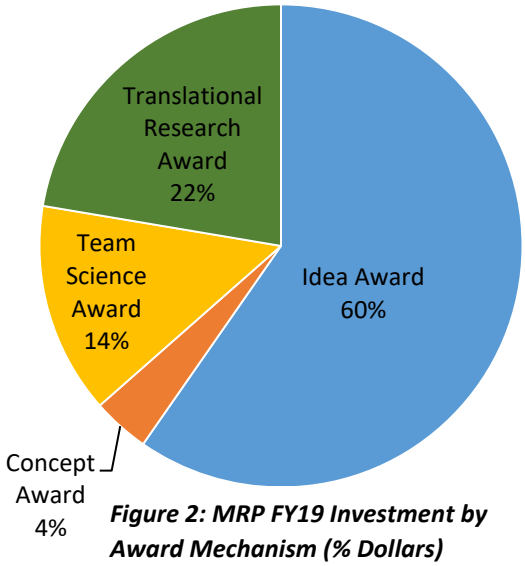


Figure 2: MRP FY19 Investment by Award Mechanism (% Dollars)

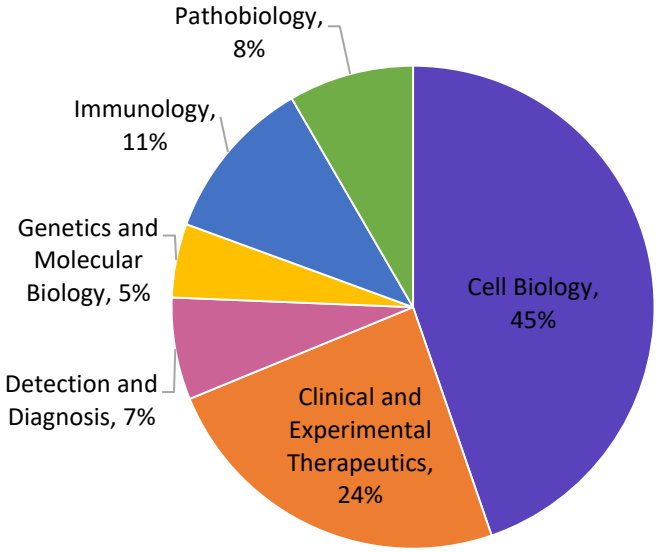


Figure 3: MRP FY19 Investment by Scientific Classification System Codes (% Dollars)

In FY20 the MRP required applicants to respond to new Focus Areas (Table XI). Researchers could propose projects in all FY20 MRP Focus Areas in the five award mechanism offered. The Technology Development Partnership Award required researchers to respond to a specific FY20 MRP Focus Area: Bioengineering (e.g., computational, imaging) approaches to address diagnostics, high-risk markers, dormancy, and metastasis. Figure 4 illustrates the FY20 MRP potential investment strategy across these award mechanisms.

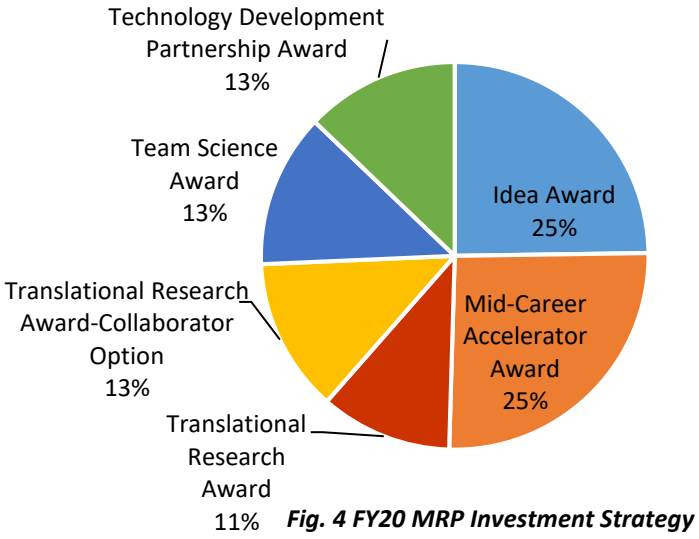


Fig. 4 FY20 MRP Investment Strategy (% of Research Budget)

Table XI: FY20 MRP Focus Areas Fig 1. FY19 Investment by Award Mechanisms (% Dollars)

Award Mechanism	Focus Areas

• Idea Award
• Mid-Career Accelerator Award
• Translational Research Award
• Translational Research Award - Collaborator Option
• Team Science Award
	• Prevention of melanoma initiation factors (e.g., UV radiation)
	• Prevention of melanomagenesis and precursor lesions (e.g., novel genetic and epigenetic drivers, oncogene induced senescence)
	• Understanding the tumor microenvironment
	  ○ Primary Tumor
	  ○ Regional Nodes
	  ○ Distal Nodes
	• Bioengineering (e.g., computational, imaging) approaches to address diagnostics, high risk markers, dormancy, and metastasis
	• Therapeutic Prevention (e.g., interruption of disease progression, recurrence)
	• Minimal Residual Disease (e.g., chemoprevention, micro-metastasis)

• Technology Development Partnership Award	• Bioengineering (e.g., computational, imaging) approaches to address diagnostics, high risk markers, dormancy, and metastasis

Peer Reviewed Cancer Research Program

Before the establishment of the MRP, melanoma (and other skin cancers) (MOSC) was a Topic Area under the Peer Reviewed Cancer Research Program (PRCRP). From FY09-FY18, the PRCRP invested a total of $54.3M in melanoma research across the Common Scientific Outline (CSO) (Figure 5). The CSO is a classification system organized around six areas of scientific interest specific to cancer research: biology; etiology; prevention; early detection, diagnosis and prognosis; treatment; and cancer control, survivorship, and outcomes. The CSO classification system allows for public, non-profit, and government agencies to compare and contrast research portfolios and promote a synergistic approach to investment in cancer research.

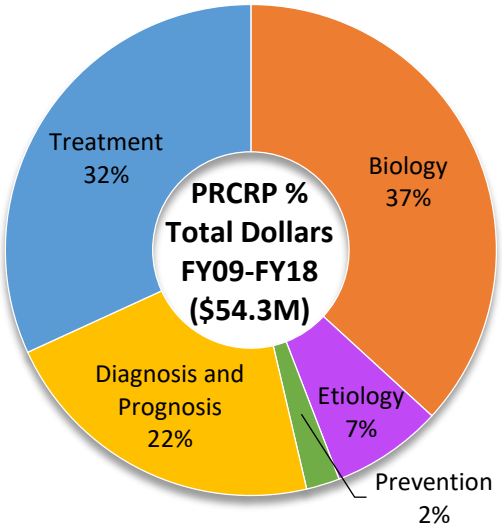


Figure 5: PRCRP Investment by CSO Coding for Melanoma Research

The PRCRP utilized multiple types of award mechanism to fund a variety of melanoma research (Figure 6). Through these different award mechanism, the PRCRP invested in developing novel ideas, translational studies, and new investigators to advance the understanding of MOSC.

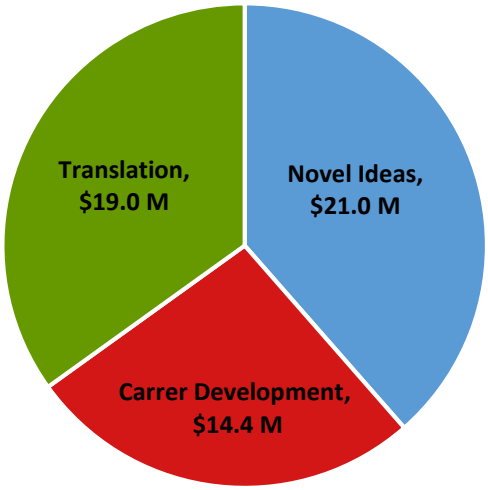


Figure 6: PRCRP Award Mechanism Total Investment in Melanoma

OTHER FUNDING AGENCIES: FEDERAL AND NON-FEDERAL

National Cancer Institute

In 2014 the U.S. Department of Health and Human Services developed a "Call to Action to Prevent Skin Cancer."[201] The National Cancer Institute (NCI) together with the Centers for Disease Control and Prevention, National Institutes of Health, and the FDA focused on measurable outcomes to include reducing melanoma mortality, reducing reported sunburn cases, reducing the number of adults and young adults that report use of artificial UV light for tanning, and increasing the proportion of adults and young adults that take protective measures to lessen the risk of skin cancer and melanoma. The NCI supported approximately 1,600 intramural and extramural research projects totaling over $590M related to melanoma and other skin cancers research in 2013-2017, as reported on the NIH RePORT. The percent relevance to melanoma research reported here is at least 50%. Figure 7 shows that for FY14-FY19 the NCI has a relatively balanced portfolio across the six scientific classification areas.

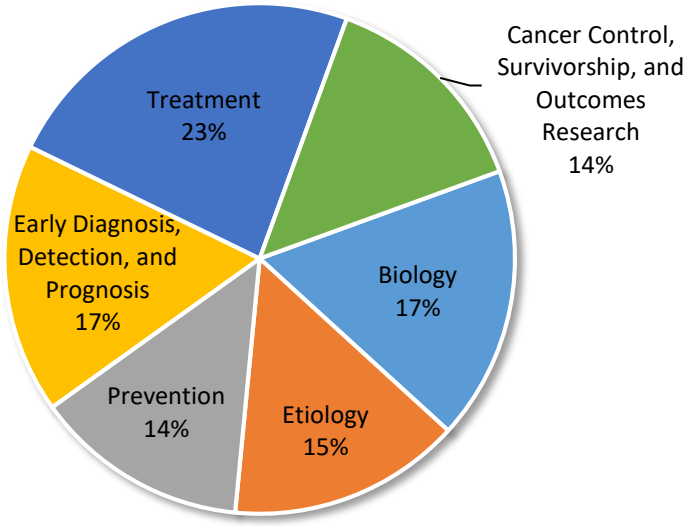


Figure 7: FY14-FY19 NCI Investment by CSO (% Dollars)

U.S. Department of Veterans Affairs (VA)

The VA reports its funded research through the Federal RePORTER. During 2014-2019 the VA has funded approximately 113 research grants, totaling over $16M, that included melanoma research. The VA invests heavily in understanding the biology of melanoma (Figure 8).

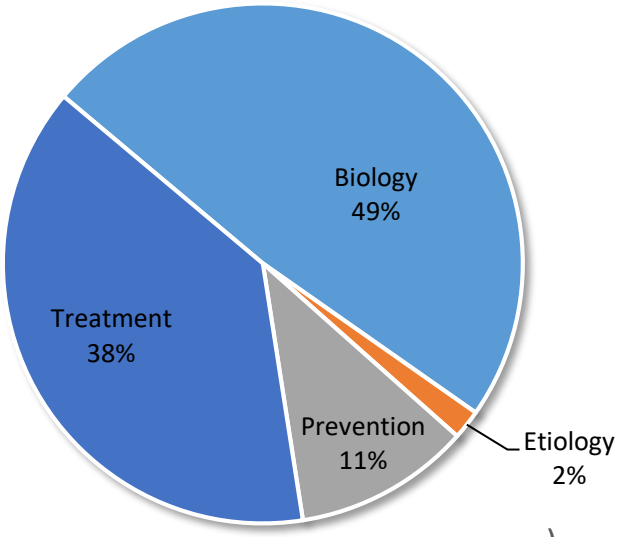


Figure 8 FY14-FY19 VA Investment by CSO (% Dollars)

Melanoma Research Alliance

The Melanoma Research Alliance[202] (MRA) is a non-profit private funder of melanoma research. Since its inception in 2007, the MRA has committed a total of $123M to melanoma research across six major award mechanism (Young Investigator Awards, Pilot Awards, Established Investigator Awards, Team Science Awards, Partnership Awards, and Dermatology Fellowship Awards). Research funded through these award mechanisms focuses on prevention, diagnosis, and staging, as well as the treatment of melanoma, including research in biological causes of carcinogenesis, skin screening, biomarkers, imagining, immunotherapy, molecular targeted therapy, and combination therapy. The majority of MRA funds are allocated for melanoma treatment. Figure 9 illustrates that from FY14-FY19 over 50% of MRA's research investment budget was focused on melanoma treatments.

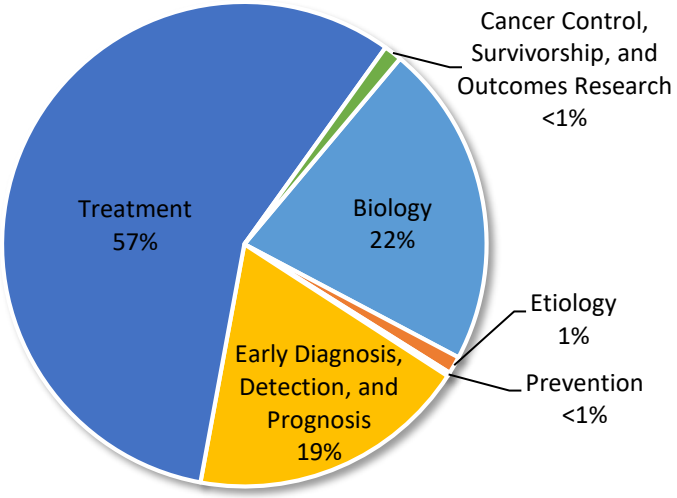


Figure 9: FY14-FY19 MRA Investment by CSO (% Dollars)

Melanoma Research Foundation

The Melanoma Research Foundation[203] (MRF) is an independent non-profit organization devoted to accelerating medical research in melanoma. Specifically, the MRF is committed to fund medical research that supports effective treatments that ultimately lead to a cure for melanoma. To reach their goals, the MRF provides a variety of grant opportunities including: Medical Student, Career Development, Established Investigator, Team, and Clinical Trial grants. In addition, the MRF plays a leading role in many scientific initiatives including: the MRF Breakthrough Consortium (MRFBC) and the creation of Community United for Research and Education of Ocular Melanoma (CURE OM), as well as initiatives that focus on rare melanoma subsets such as pediatric and mucosal. Since its establishment in 2008, the MRF has allocated over $19.6M to melanoma research spanning 78 institutions in 30 states. From FY14-FY19, approximately 50% of MRF's research investment budget was focused on the biological mechanism of melanoma (Figure 10).

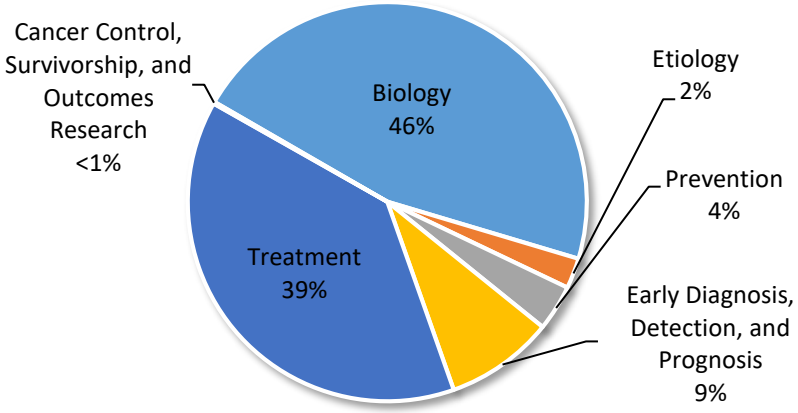


Figure 10: FY14-FY19 MRF Investment by CSO (% Dollars)

AIM at Melanoma

Founded in 2004, AIM at Melanoma[204] is a global foundation dedicated to discovering more effective treatments for melanoma. By bringing together leading melanoma researchers their collaborative research

through the International Melanoma Tissue Bank Consortium, AIM at Melanoma believes that a cure for melanoma will be discovered. There are currently six branches participating in the International Melanoma Tissue Bank Consortium: H. Hillman Cancer Center, University of Pittsburg Medical Center (UPMC); Knight Cancer Institute, Oregon Health and Science University (OHSU); California Pacific Medical Center (CPMC); Robert H. Lurie Comprehensive Cancer Center, Northwestern University; Peter MacCallum Cancer Centre, Victoria, Australia; and the Alfred Hospital, Melbourne, Victoria, Australia. To date, AIM at Melanoma has raised more than $1.5M toward the International Melanoma Tissue Bank Consortium.

The Society of Melanoma Research

The Society of Melanoma Research[205] (SMR) is an all-volunteer group of scientists working to discover the mechanisms responsible for melanoma, and ultimately new therapies. The SMR brings together researchers in a noncompetitive manner to unite the melanoma scientific community. The overarching goal of the SMR is to bring together members with varying backgrounds, from basic researchers to translational researchers to clinicians. The SMR accomplishes this by supporting young investigators interested in establishing a career in melanoma researcher through travel awards.

International and Other Funding Organizations

Outside of the US, Australia (AU) is one of the largest funders of melanoma research. Over the last decade Australia has funded 156 clinical trials in melanoma. In 2020, melanoma became the third most commonly diagnosed cancer in AU, behind prostate cancer and breast cancer.[206] It was estimated that during 2020 a total of over 17,000 Australians would be diagnosed with melanoma. The reason for this high incidence is likely not linked to a single cause, but a combination of risk factors (see Risk Factors section). As a result, the majority of AU's melanoma funding is in treatment (Figure 11).

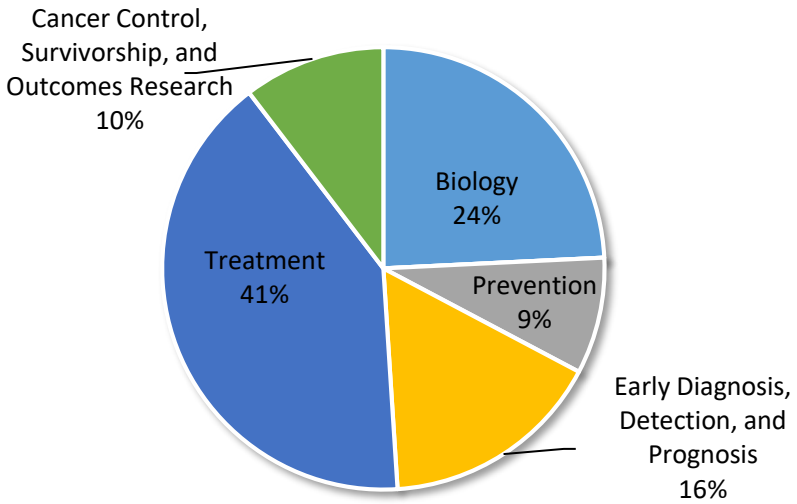
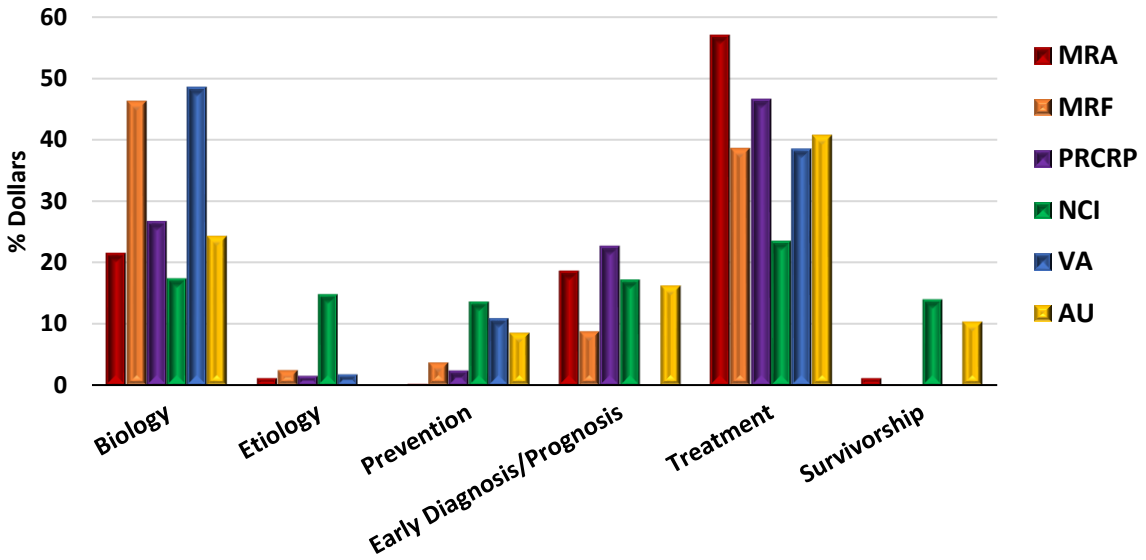


Figure 11: Australia Investment in Melanoma Research by CSO (% Dollars)

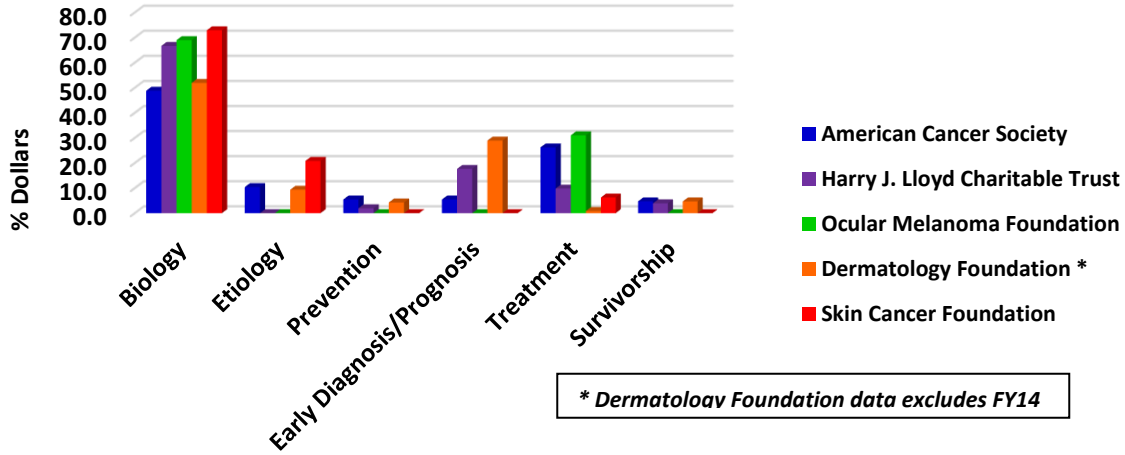
Figures 12 shows that, similar to US funding organizations, Australian organizations primarily focus on treatment modalities for melanoma.

Figure 12: FY14-FY19 Investment by CSO (% Dollars)



There are a number of other foundations, outlined below, that support melanoma research. Figure 13 shows the majority of research is in understanding melanoma biology followed by treatment. The Skin Cancer Foundation,[207] established in 1981, has been awarding research grants to physicians and investigators to support pilot research projects related to prevention, detection, and treatment of skin cancer. The Dermatology Foundation[208] is dedicated to enabling advancements in patient care by providing research funding to early and mid-career investigators. Since its founding in 2003, the Ocular Melanoma Foundation[209] has raised nearly $2M to support ocular melanoma patients and provide funding for ocular melanoma research. The Tara Miller Melanoma Foundation[210] has funded over $4M in melanoma research, covering 29 projects and 10 clinical trials. The Harry J. Lloyd Charitable Trust[211] has provided more than $17M in funding for 137 melanoma research grants in one of three areas: career development, basic science, and translational research.

Figure 13: FY14-FY19 Investment by CSO (% Dollars)



When dollars invested in melanoma research among Federal, non-Federal, and international funders are combined based on CSO research areas it is apparent that there is a lack of funding in melanoma prevention compared to the other research areas (Figure 14).

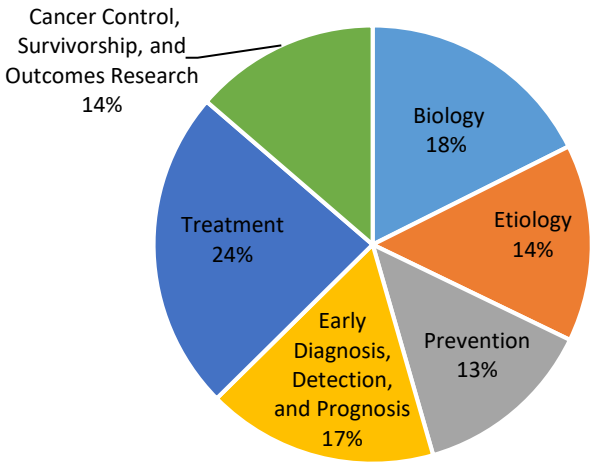


Figure 14: FY14-FY19 Federal, non-Federal, and International Investment in CSO (% Dollars)

The lack of funding in melanoma prevention has prompted the MRP to challenge the melanoma research community to look beyond sunscreens and protective clothing as preventive measures against melanoma and to redefine prevention to include the entire melanomagenesis process. The MRP realizes investing in research along each step of the melanomagenesis process is an opportunity to prevent the metastatic effects of melanoma.

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