Chapter 1
Melanoma: Historical Context

Suraj Venna, Sekwon Jang and Michael Atkins

Abstract We are in the midst of a therapeutic revolution for patients with melanoma. This chapter reviews several topics on melanoma from epidemiologic trends, to the evolution of the surgical approach, to adjuvant treatment of melanoma, and also reviews various systemic therapies for metastatic melanoma. Each component of this chapter describes advances from a historical perspective, beginning with the first descriptions of melanoma in the literature, to the discovery of activating B-raf mutations in melanoma, and concluding with the current immune and targeted based therapies for advanced melanoma. It serves as a segue to the more detailed therapies and advances in the ensuing chapters.

Keywords B-raf · Checkpoint inhibition · Adjuvant therapy · Chemotherapy · Biochemotherapy · Immunotherapy · MC1R · Risk factors · Sentinel lymph node biopsy · Vaccines

1.1 Introduction

John Hunter in 1787 excised a tumor from the jaw of a young man and aptly described it as a "*cancerous fungous excrescence.*" Hunter detailed that the tumor recurred on the patients chin several years later, thought to perhaps have been incited by trauma as this young gentleman had partaken in a bar room brawl at that time. This specimen was preserved for nearly 200 years in the Hunterian Museum of the Royal College of Surgeons in London and is now specimen number 219 [1]. In 1968 the specimen was examined and verified to be melanoma. Rene Laennec in 1806 is credited as the first physician in modern times to describe melanoma as a disease and published this while still a medical student [2]. William Norris in 1820

M. B. Atkins (✉)
New Research Building, Rm E501, 3970 Reservoir Rd, NW, Washington DC 20056, USA
e-mail: mba41@Georgetown.edu

S. Venna · S. Jang · M. B. Atkins
Medstar Washington Cancer Institute, Lombardi Comprehensive Cancer Center, Medstar Georgetown University Hospital, Washington DC, USA

R. J. Sullivan (ed.), *BRAF Targets in Melanoma,*
Cancer Drug Discovery and Development 82, DOI 10.1007/978-1-4939-2143-0_1

published his post-mortem description of a patient with atypical nevi who developed and died of metastatic melanoma: "*On making an incision through the original tumour, I found the texture to be heterogeneous; it was of a reddish and whitish brown tint throughout, not very unlike the internal structure of a nutmeg. The newly formed tumour, and the tubera around, though during life they wore a very different aspect, after death both exhibited the same dark-coloured appearance. On puncturing a considerable number of the different tubercles, a thick dark fluid was discharged from them* [3]." The first formal acknowledgement that melanoma, in advanced stages, is untreatable and a death sentence, was documented in 1844 by Samuel Cooper in his textbook *First lines of theory and practice of surgery* [4]. He published that the only chance for survival was early removal of the disease, stating that "*No remedy is known of, for melanosis….the only chance of benefit depends upon the early removal of the disease by operation….*" However, the earliest example of melanoma has been suggested to come from Mummified skeletal remains of Peruvian Incas dating to 2400 BC [5]. Over time, we have moved from simple descriptive terms such as *cancerous fungous excrescence*, to defining the molecular pathways responsible for the development of melanoma. This has elegantly been now translated into targeted therapies for melanoma that provide the expectation of controlling this often devastating disease in significant subsets of patients for increasingly extended periods of time.

After a nearly 15 year stand-still for the treatment of metastatic melanoma, we are in the midst of a virtual revolution in systemic treatment of patients with melanoma. The targeted therapy era for melanoma was initiated by the finding that a significant proportion of melanomas carry activating mutations in a component of the mitogen activated protein kinase (MAPK) signaling pathway. Activating mutations in B-raf were identified in 2002 and also found to occur in the vast majority of benign nevi (80%) [6, 7]. These findings led to a resurgence of interest in melanoma but also led to continued interrogation of the MAPK pathway and other pathways. The discovery of B-raf mutations in benign nevi made it clear that this may be a necessary but early step in melanoma progression and other critical targets must also be involved.

1.2 Epidemiologic Trends

Melanoma accounts for 5% of all skin cancers but is the major cause of death from skin cancer. In the year 2013, there were an estimated 76,690 new cases of invasive melanoma in the United States and over 9480 deaths attributable to melanoma [8]. This equates to one melanoma-specific death every hour. The number of annual new cases is likely underestimated given that in-situ lesions and thin invasive melanomas (Stage 1a) are not consistently reported to tumor registries, being excised in the outpatient and private practice settings [9]. The lifetime incidence of developing melanoma in the United States was 1/1500 for individuals born in the early 1900's [10]. Between 1950 and 2000, there was an explosive increase in melanoma

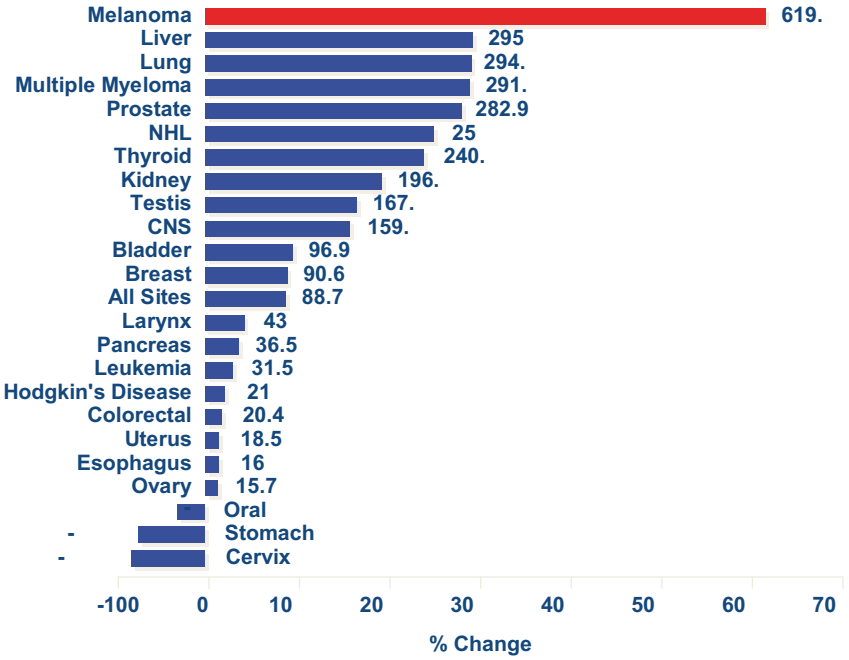

Fig. 1.1 Trends in cancer incidence SEER 1950–2000. (From http://seer.cancer.gov/archive/csr/1975_2000/results_merged/topic_inc_mor_trends.pdf)

incidence rate, outpacing all other tumors with a 619 % increase during this 50-year interval (see Fig. 1.1). Today it is predicted that the lifetime incidence of developing invasive melanoma for a white man or woman is 1/50 or 2 % of the population. All thicknesses of melanomas have contributed to this increased incidence [11]. During a 14 year period from 1992–2006, the annual rise in melanoma incidence was over 3 % in non-hispanic whites [12]. Over the last 10 years the annual rise in cases has been about 2.6 % [13]. Interestingly, death rates over the same period have remained stable (Fig. 1.2; [13]). It has been suggested that this rise in incidence may be due to diagnostic drift with a lower threshold for diagnosing melanoma histologically [14, 15]. However, incidence trends have found increases not just in thin melanomas, but also in thicker melanomas, for which diagnostic drift would be less likely [16].

Melanoma ranks 2nd only to leukemia in terms of years of productive life years lost (YPLL) [17]. A recent SEER analysis studied incidence trends of melanoma in young adults for the period of 1973 thru 2004. Age-adjusted annual incidence of melanoma among young men increased from 4.7 cases per 100,000 persons in 1973 to 7.7 per 100,000 in 2004. Among women, age-adjusted annual incidence per 100,000 increased from 5.5 in 1973 to 13.9 in 2004 [18]. Given that melanoma preferentially affects those during the most productive years of life there is a societal burden associated with this disease that exceeds its incidence. On average, an individual in the United States loses 20.4 years of potential life during

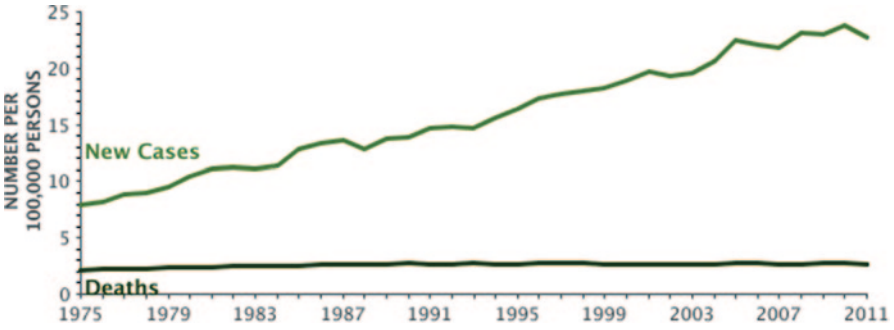

Fig. 1.2 During this period, new cases of melanoma increased significantly while death from melanoma remained relatively constant (From SEER Website: http://seer.cancer.gov/statfacts/html/melan.html)

their lifetime as a result of melanoma mortality compared with 16.6 years for all malignant cancers [19]. Among studies examining all stages of melanoma, annual treatment costs ranged from $ 44.9 million among Medicare patients with existing cases to $ 932.5 million among newly diagnosed cases across all age groups [20]. Melanoma mortality significantly impacts the US economy with a loss of $ 3.5 billion annually [19]. Given the substantial costs of treating melanoma, public health strategies should include efforts to enhance both primary prevention (reduction of ultraviolet light exposure for example) and secondary prevention (earlier detection) of melanoma.

Risk factors There are many risk factors for melanoma including phenotype, genotype, family history, and exposure to ultraviolet light (UVL) with varying effects on the relative risk of developing melanoma (Table 1.1). The focus on risk factors stems from awareness of increased risk, based on the host phenotype, such as those with fair skin, atypical moles and family history, which cannot be altered, to those risks which can be modified such as exposure to UVL, whether from natural or artificial sources. Up until recently, we relied heavily on indirect evidence of UVL being an important factor in the genesis of melanoma. In 2009, the first comprehensive analysis of the melanoma genome was undertaken based on the assessment of an immortalized melanoma cell line, COLO-829, derived from a 43 year old man who died of metastatic melanoma, from an unknown primary [21]. Over 33,000 somatic mutations were identified, including mutational signatures of UVL. Of the 510 dinucleotide substitutions, 360 were CC>TT/GG>AA, changes associated with UVL exposure. The risk factor of UVL exposure also relates to intrinsic factors that increase risk, such as variants in the melanocortin-1 receptor (MC1R), responsible for determining skin pigmentation and processing of UVL-induced skin damage [22]. Certain MC1R polymorphisms are associated with an increased risk of melanoma, and are considered low-penetrance melanoma susceptibility alleles [23, 24]. In one study, MC1R variants were associated with melanoma progression and thicker melanomas in both cases of sporadic and familial melanoma [25]. It has been shown that the more variants of MC1R a patient has, the greater the likelihood

Table 1.1 Relative risk of melanoma

Fair skin	2–18
Freckles	3–20
Blonde hair	2–10
Red hair	2–6
Inability to tan	2–5
Blue eyes	2–5
Constant sun	2–5
Intermittent sun	2–3
Immunosuppression	2–8
Tanning bed use	2–4
NMSC	3–17
Personal history of melanoma	9–10
Family history of melanoma	8
50–100 common nevi	2–64
1 or 2 atypical moles	2–11
Atypical mole syndrome patients	
No personal or family history of melanoma	2–92
Personal history, but no family history	8–127
1 family member with melanoma	33–444
2 family members with melanoma	85–1269

of their melanoma harboring a mutant B-Raf [26]. The MC1R-Braf association is now well established, with MC1R variants demonstrating an increased risk of B-Raf mutant melanomas based on having one or two variants [26, 27]. Multiple MC1R variants had up to a 15-fold increased risk of developing B-Raf mutant melanoma. There was no association between MC1R status with melanomas without B-Raf mutations. The mechanism behind this association remains to be elucidated.

Screening The goal of primary prevention is to prevent the development of a disease and in the case of melanoma, this may be accomplished by limiting UVL exposure. However, secondary prevention may be a more realistic approach to detect melanomas at early stages and cure the patient with a simple excision. The United States Preventative Services Task Force (USPSTF) last reviewed evidence for skin screenings in 2009 and they concluded that the evidence for or against total skin exams for the early detection of melanoma and non-melanoma skin cancer was insufficient, due mostly to limited high quality studies and lack of randomized controlled trials (RCT's) [28]. However two recent studies from Germany have reignited the debate and make the case for population based skin cancer screenings. One was an observational study involving over 360,000 screened participants from 1 region of Germany. The mortality rates were compared with 3 adjacent townships and the country of Denmark. The screened population demonstrated a 47% decrease in melanoma mortality after this population-wide skin cancer screening

program when compared to the 4 other regions devoid of such a screening intervention [29]. In another study, the SCREEN (Skin Cancer Research to provide Evidence for Effectiveness of Screening in Northern Germany) project, melanoma incidence was determined before, during, and after skin cancer screening in the German state of Schleswig-Holstein [30]. The incidence of melanoma increased in both men and women during this screening effort: invasive melanoma in men: +4.0 per 100,000 (95% CI: 1.6; 6.4); women: +8.9 per 100,000 (95% confidence intervals (CI): 6.1; 11.7); and decreased afterwards (women: −10.6 per 100,000 (95% CI: −13.3; −7.9); men: −4.1 per 100,000 (95% CI: −6.5; −1.7). During that same period of time, these trends did not occur in another German state where the screenings were not being performed. On a practical level, RCT's for melanoma screening would be a large and at this time unrealistic undertaking. In order to prove that population based skin screenings can affect mortality from melanoma, it has been estimated that such a RCT would necessitate 800,000 screenings to generate adequate power to arrive at such conclusions [31]. Until such RCTs are completed, it should be duly noted that in the case of melanoma, screening strategies as a form of secondary prevention has value in that melanomas can be readily identifiable by educated patients and primary care providers. Unfortunately, it is estimated that only 30% of dermatologists perform full skin screenings [32]. Finally, although dermatologists are the group best trained at identifying skin cancers, there is a shortage of these physicians in the workforce relative to the population [33–35]. Therefore it is important to identify those individuals at highest risk of developing melanoma and focus screening efforts among these groups. These would be people with a personal and/or family history (PH/FH) of melanoma as well as those with a phenotypic risks such as high nevus count, atypical nevi, fair skin; behavioral risks such as tanning bed use and indiscriminate UVL exposure; and those who have had non-melanoma skin cancers (NMSC), such as basal cell carcinoma and squamous cell carcinoma. In one study, those with a history of NMSC had a 17-fold increased risk of developing melanoma, with an average follow-up of over 9 years [36]. The vast majority of the melanomas in this study were detected on sun-protected sites with an average Breslow's depth of 0.70 mm. It is important to remember that about 50% of newly diagnosed melanomas are detected by the patient upon the Self Skin Examination (SSE) [37, 38]. Therefore, physicians should continue to alert those at high risk and educate patients on what to look for. Although a significant portion of melanomas are detected by the patient, when melanomas are detected by dermatologists, they are significantly thinner than those detected by patients performing the SSE [39, 40].

Examination by a physician allows for anatomic sites to be evaluated that would be difficult for the patient, such as the back, scalp, and calves. Total body photography (TBP) is an important adjunct in the surveillance of patients to document stability of nevi and also to identify new lesions [41]. TBP has allowed for the detection of early stage melanomas that may otherwise have been missed and also allow the clinician to avoid unnecessary biopsies of benign lesions that are documented to have remained stable during follow up exams [42, 43]. Unfortunately, due to the related infrastructure for storage of digital images and retrieval, TBP is infrequently

used in private offices. In a recent survey of US dermatology training programs, the most common reasons for not using TBP were logistical and financial [44]. The most popular non-invasive bedside tool to evaluate nevi is surface microscopy or dermatoscopy. This is a hand-held device comprised of a series of non-polarized lights with magnification of 10x. The clinical diagnosis of melanoma with the unaided eye has a sensitivity of about 60%. This can be significantly enhanced with dermatoscopy.

Dermatoscopy improves the ability to diagnose melanoma, and correctly identify benign skin lesions and forego unnecessary skin biopsy [45–47]. However, specialized training is needed in the use of this bedside aide [48]. In comparison to the unaided eye, dermatoscopy improves sensitivity by 20% and specificity by 10% [49]. Other techniques to improve early detection of melanoma are also being investigated. Computer assisted systems based on multi-spectral image analysis are available. The machine will image a mole or suspicious lesion and calculate a number of parameters such as color gradient, width, borders, and other morphologic features and then compare it to a database to determine if the combined parameters reach a threshold for melanoma, and therefore to biopsy. These types of devices based on pattern recognition have pitfalls such as in the atypical nevus patient. In a patient with atypical nevi, the computer-generated pattern can yield a worrisome score requesting biopsy, when in reality the patient has 50 other nevi with a similar pattern. This underscores a very important clinical principle when evaluating patients with many nevi, especially atypical: proceed with caution when considering skin biopsy and avoid making that decision on whether or not to biopsy a suspicious mole in isolation. In other words, that decision should be done in the context of multiple factors including the patient's phenotype and other risk factors. Confocal laser scanning microscopy (CLSM) is another method to evaluate nevi in a non-invasive manner where horizontal sections are visualized to a depth of the papillary dermis. Due to the fact that melanin and melanocytes offer strong contrast with this near-infrared device, it would theoretically be an effective technique for cutaneous melanoma diagnosis [50]. In one study CLSM had a higher sensitivity than dermatoscopy, but a lower specificity for diagnosing melanoma [51]. However the cost, time to image lesions, and most importantly the extensive training needed to capture and interpret images are major barriers to its widespread use. At the current time, CLSM is relegated to a few academic centers with very limited clinical utility. On a practical level, the clinical examination complemented with dermatoscopy should be considered the standard method to evaluate patients at risk for melanoma.

1.3 Evolution in Surgical Management

The nature of the extent of surgery has evolved slowly over the centuries as the understanding of the biology of melanoma has progressed. William Handley is credited with setting the course for the surgical treatment of melanoma for a 50-year period. In 1907 he advised that melanomas should be excised with 5 cm margins

down to the level of the fascia and that the regional nodes should be removed, sometimes termed in those days as *lymph node evacuations* [52]. In 1967, a Finnish surgeon, Grete Olsen, published data that she gathered from the Finsen Institute and Radium Center Copenhagen, Denmark, on 500 melanoma patients [52]. Metastases to regional nodes developed more frequently in those patients in whom the underlying fascia had been removed versus those in whom the fascia was intact, 45% versus 8–14%. She stated in this paper that "*when the fascia is excised ….there is now nothing to hinder the spreading of melanoma cells from the subcutis region to the deep subfascial lymphatic vessels*…." The theory was that the removal of the muscular fascia propagated metastatic melanoma, in that the fascia had a physical barrier role that would otherwise obstruct melanoma from metastasizing. And since Olsen's publication, the depth of melanoma excision has been through the subcutis with preservation of the muscular fascia. There have never been studies examining the optimal depth of excision for melanoma. The radial margins of excising melanoma have been reduced considerably since the initial guidelines set out by Handley in 1907. The margins of excision now recommended are designed to limit the risk of local recurrence with its potential effect on survival by capturing, in-theory stray melanoma cells with the radial margin. These margins are modified according to particular anatomic site. However, the guidelines for margins of excision for melanoma are based primarily on 5 multi-institutional trials, which compared excision of margins of 1 vs. 3 cm (2 studies), 2 vs. 4 cm (2 studies), and 2 vs. 5 cm (1 study) [53–57]. The wider margins did not improve overall survival; however, the current practice is to do no less than 1 cm resection margin for melanomas less than 1 mm in depth; 1–2 cm for melanomas measured between 1–2 mm; and at least 2 cm for melanomas measuring 2.01 mm or greater in thickness. The final exact margin is always decided in the context of the individual patient and anatomic location of the melanoma. The current evidence is insufficient to address the optimal excision margins for melanoma and less is known regarding the optimal depth of excision. However, it is clear at least, that very wide margins of 5 cm do not offer patients a survival advantage.

For over 100 years, radical en-bloc or 'gland excision' was carried out, advocated and published first by Dr. Herbert Snow in his lecture titled "Melanotic cancerous disease" in the Lancet, presented at the Cancer Hospital in London, February 5, 1892 [58]. In this published lecture, he aptly noted that the melanomas could arise from pre-existing nevi stating, "non-prominent moles or cutaneous stains may be antecedent to melanotic developments." And also assumed that the progression of melanoma was from skin to lymph nodes to systemic, and therefore advocated for complete node dissection along with wide local excision: "it is essential to remove, whenever possible, those lymph glands which first receive the infective protoplasm."

Once it became clear that the level and absolute depth of invasion of melanoma are key determinants of prognosis and likelihood of occult nodal disease, the role of these radical 'gland excisions' was called into question and ultimately evolved into a dramatic change in the approach to the assessment of nodal disease. In a paper published in 1979 titled "Melanoma Thickness and Surgical Treatment" the authors

set out to examine their experience with Stage 1 patients to determine the role of elective node dissection [59]. They found that melanoma thickness correlated with risk of nodal disease, with 62% occurrence in melanomas greater than 4 mm, 57% in lesions between 1.50–3.99 mm, 25% in lesions between 0.77–1.49 mm, and 0% in melanomas less than 0.77 mm. At that time, they concluded that at least in the thin melanomas (<0.77 mm) elective lymph node dissection (ELND) was not justified. However, they recommended continued ELND for intermediate thickness melanomas 1.50–3.99 mm based on the significant difference in 5-year survival (83% WLE + ELND vs. 37% WLE alone). Interestingly, they also commented that for melanomas >4 mm, "the potential benefits of immediate lymphadenectomy are much less because the incidence of simultaneous metastases at distant sites appear to diminish the beneficial effects of removing any regional metastases."

Dr. Donald Morton is credited with developing the technique of sentinel lymph node biopsy (SLNB) for melanoma, a minimally-invasive way to stage the regional nodes, and better stratifying patients into those who may benefit from a subsequent complete node dissection, thereafter. Since thick melanomas have a propensity for hematogenous spread, the largest prospective trial assessing the value of sentinel lymph node biopsy (SLNB) for melanoma focused primarily on intermediate thickness melanomas. A "final" analysis of the largest trial assessing the role of SLNB for intermediate thickness melanomas showed that there was no significant difference in the 10-year melanoma-specific survival when comparing those patients with or without sentinel lymph node procedures [60]. However, there was a significant improvement in the 10-year disease free interval in the SLNB group versus the observation group among patients with intermediate-thickness melanomas, defined as 1.20–3.50 mm (71.3 ± 1.8% vs. 64.7 ± 2.3%; hazard ratio for recurrence or metastasis, 0.76; $P = 0.01$), and those with thick melanomas, defined as >3.50 mm (50.7 ± 4.0% vs. 40.5 ± 4.7%; hazard ratio, 0.70; $P = 0.03$). In the node positive patients, those who were diagnosed via SLNB as compared to macroscopic presentation (the observation group), the 10-year melanoma-specific survival rate was 62.1 ± 4.8% versus 41.5 ± 5.6% in the observation group (hazard ratio for death from melanoma, 0.56; 95% CI, 0.37 to 0.84; $P = 0.006$). The final analysis established unequivocally, that (1) SLNB is accurate and provides prognostic information; (2) early intervention decreases the risk of nodal recurrence, distant metastases, and death from melanoma; (3) SLNB can identify patients with nodal disease who may benefit from immediate completion lymphadenectomy.

The role of surgery in patients with late stage melanoma continues to evolve especially in the current era, given the number of systemic treatment options that have recently become available. Recent data has also supported a limited role of metastasectomy. A SEER analysis of Stage IV patients undergoing metastasectomy found that patients who underwent metastasectomy (33.6%) had an improved median (12 months versus 5 months) and 5-year overall survival (16% versus 7% ($P < 0.001$) as compared to patients who did not [61]. In patients with M1a disease ($n = 1994$), this improvement of survival following metastasectomy was enhanced; median survival of 14 months versus 6 months and 5-year overall survival of 20% versus 9% ($P < 0.001$).

The surgical management of melanoma has been steadily refined over the past century, with research efforts until recently having the luxury of being largely unencumbered by the confounding effects of effective systemic treatment approaches. With the advent of systemic therapies that unequivocally prolong survival in patients with stage IV melanoma, the integration of surgery with other effective treatment will likely need to be more actively considered.

1.4 Adjuvant Therapy

For the majority of patients presenting with melanoma, complete surgical excision will be possible and potentially curative. However, the risk of systemic recurrence is high among patients with thick primary lesions or positive lymph nodes. There has been considerable effort to assess adjuvant interventions including adjuvant chemotherapy, nonspecific immunostimulants or vaccines. However, none of these approaches, used either alone or in various combinations, proved beneficial when compared to either observation or placebo in randomized clinical trials. Adjuvant immunotherapy with high dose interferon alpha (IFNa) prolongs disease-free survival, and in some studies prevents relapse and death in as many as 25–33 % of patients at risk. High dose IFNa, and more recently pegylated IFNa received US Food and Drug Agency (FDA) approval as adjuvant treatments for patients stage IIB, IIC and III melanoma and are presently considered the standard of care. Nonetheless, a recent National Cancer Data Base analysis of over 34,000 patients with Stage III melanoma, suggest that less than one-third of patients eligible for such adjuvant treatment actually receive it [62]. Thus, there remains a need to develop adjuvant treatments with improved efficacy and/or reduced toxicity that can achieve general acceptability.

Cytotoxic Chemotherapy and Combination Chemotherapies Single-agent chemotherapy or combination chemotherapy regimens have been evaluated for the adjuvant treatment of patients with melanoma. In a randomized controlled trial, the administration of dacarbazine (DTIC) either alone or in combination with BCG after wide local excision and regional lymphadenectomy failed to show improvement in disease-free survival (DFS) or overall survival (OS) [63]. The combination chemotherapy regimen of carmustine, actinomycin-D, and vincristine administered for 6 months was compared to observation among 173 patients with resected stage III or stage IV melanoma [64]. This trial demonstrated a significant improvement in relapse-free survival (5-year Kaplan-Meier estimates of relapse-free survival of 29 % vs. 9 %; $p = 0.03$), however, there was no difference in overall survival. Given the small size of this trial and the lack of confirmatory results in larger trials, adjuvant chemotherapy is not currently advocated for treatment of patients with high-risk melanoma.

Nonspecific Immunostimulants and Vaccines Multiple different immunostimulant and vaccine strategies have been pursued as adjuvant therapy for patients with high

risk melanoma over the past 40 years with none showing convincing or reproducible benefits. Some of the most promising of these approaches are described below.

Observation of regression in intradermal metastases of melanoma after intralesional injection of Bacillus Calmette-Guerin (BCG) led to adjuvant trial with BCG in high-risk patients [65]. In the EORTC 18781 trial, 353 patients were randomized to two different BCG preparations or to follow-up only [66]. Although the treatment was generally well tolerated, there was no benefit in patient survival and time to relapse. *Corynebacterium parvum* is another micro-organism which stimulates the immune system. In a randomized clinical trial of *C. parvum* compared to observation in 260 patients with clinically localized melanoma, there was no significant difference in survival between the two treatment arms [67]. Levamisole, an antihelminthic agent with immunomodulatory effects, was tested in a few randomized controlled trials. It failed to show any benefit in all except one study. This study demonstrated statistically insignificant reduction in the death rate and the recurrence rate in levamisole group compared with observation [68]. Levamisole has never been adopted widespread as a therapeutic agent.

In the wake of negative studies with nonspecific immunostimulants, investigators switched course and attempted to develop vaccines capable of eliciting a specific host immune response against melanoma. A variety of vaccination strategies using autologous or allogeneic melanoma cells have been tested over the last few decades. Technical complexities inherent in harvesting tumor and preparing a vaccine made it difficult to test autologous cellular vaccine in large multi-institutional trials. Allogeneic tumor cell vaccines, conversely, are generally prepared from cultured cell lines or lysates allowing the conduct of large-scale, multi-institutional clinical trials. The Southwest Oncology Group (SWOG) conducted one such, large randomized trial of an allogenic melanoma vaccine (melacine) compared to observation in patients with intermediate-thickness, node-negative melanoma [69]. There was no evidence of improved disease-free survival among patients randomized to receive vaccine. Canvaxin, a polyvalent cell vaccine composed of a combination of allogeneic cell lines, showed great promise in a variety of phase II trials [70]. However, it also failed to show improvement in progression-free or overall survival in randomized phase 3 trials comparing canvaxin plus BCG to placebo plus BCG in patients with resected melanoma stage III and stage IV disease [71].

Melanoma vaccines based on peptides or gangliosides also have been developed and examined in clinical trials in the adjuvant setting. The GM2 ganglioside is expressed in the majority of melanomas and could induce an antibody response. A GM2 vaccine was shown to be associated with freedom from disease recurrence in patients who developed an antibody response to the vaccine. Combining the vaccine with GM2-KLH/QS-21 adjuvant led to enhanced immunogenicity suggesting it might be an even more potent adjuvant therapy. However a randomized phase III trial comparing standard HD IFN to the GM2/KLH/QS-21 vaccine in patients with Stage IIB and III melanoma (E1694) conducted in the US Intergroup, had to be closed early because there were 50% more relapses and deaths on the vaccine arm relative to the IFNa arm [72]. In a second randomized phase II study, E2696, patients with stage III melanoma were randomized to receive two different

schedules of IFNa, IFNa + the GM2/KLH/QS-21 vaccine or the vaccine alone [73]. In this small study the two IFNa containing arms showed a significant improvement in relapse free survival (RFS) over the vaccine only arm. This same vaccine was also compared to placebo by the EORTC in a randomized Phase III trial involving 1314 patients with stage II melanoma [74]. A trend toward adverse overall survival outcome for the vaccine arm led to trial termination at the 2nd interim analysis; however, more mature data has suggested no significant difference in any outcome.

The majority of patients with melanoma have the MAGE-A3 antigen expression on the tumors and MAGE-3 vaccination is an attractive strategy. A phase I/II study demonstrated MAGE-3-specific antibody and T-cell responses following vaccination in patients with MAGE-3-positive tumors [75]. This led to a randomized phase III clinical trial (DERMA) in patients with stage III nodal metastases and detectable MAGE-3 expression in resected lymph nodes. A recent sponsor-led press release from September 2013 based on an independent analysis failed to show significant extension of DFS in Stage III patients with MAGE-A3 tumors who were on the vaccine versus placebo. However, the trial will continue to assess its second co-primary endpoint of DFS in the gene signature positive patients. Results from this analysis are expected in 2015. The National Cancer Institute surgery branch reported vaccination efforts in 95 HLA-A*0201 patients at high risk for recurrence of melanoma who received prolonged immunization with a peptide vaccine, gp100209-217 [76]. Vaccination was highly effective at inducing large numbers of self/tumor-Antigen reactive T cells, however, there was no difference in the levels of antitumor Antigen-specific T cells in patients who recurred compared with those who remained disease-free. Based on the results of this extensive research effort, one must conclude that adjuvant vaccine strategies in patients with resected high and intermediate risk melanoma have yet to show efficacy and newer approaches and a better understanding of tumor immunology are necessary to advance this field.

Interferon Type I IFNs, including IFNa, are natural proteins produced by immune cells in response to infectious agents. Durable responses seen in patients treated with IFNa for metastatic melanoma, particularly in those patients with small volume and soft tissue only disease, led to investigations in the adjuvant setting for patients with high-risk resected melanoma [77]. The majority of studies with high-dose IFNa have been conducted by the Eastern Cooperative Oncology Group (ECOG). The first trial (E1684) randomized 287 patients with resected Stage IIB or III melanoma to either observation or high-dose IFNa with an induction phase of daily intravenous IFN-a at 20 million international units (MU)/m^2 for 4 weeks followed by 48 weeks of maintenance therapy at 10 MU/m^2 subcutaneously 3 days a week [78].This study demonstrated statistically significant improvement in both relapsed free and overall survival (one-sided $p = 0.0237$) for the IFNa treated patients relative to those on observation at a median follow-up time of 6.9 years. On the basis of these results, the US FDA approved this high-dose IFNa regimen as the first postsurgical adjuvant therapy for stage IIB (T4) and III melanoma in 1996. However, the benefits of IFNa therapy on overall survival decreased, and eventually disappeared, in patients who were followed for a median of 12.6 years based on a pooled analysis [79]. This called into question the impact of high-dose IFNa

on overall survival. The controversy regarding the survival benefits of adjuvant IFNa was further heightened by subsequent ECOG led studies showing conflicting results. For example, E1690 randomized patients with Stage II and III melanoma to high-dose IFNa, lower dose IFNa or observation and showed an improvement in relapse free survival for the high-dose IFNa arm, but no difference in overall survival [80], while E1694 (as noted above) showed significant improvement in both relapse free and overall survival for high-dose IFNa compared to a ganglioside vaccine [72]. Large meta-analyses have tried to address this controversy. Mocellin et al confirmed that IFNa has a substantial, if limited, benefit [81]. This analysis, which included trials with high, intermediate, and low-dose interferon, showed an overall hazard ratio of 0.82 for relapse-free survival ($P<0.001$), with a smaller, but still significant risk reduction of 0.89 for overall survival ($P=0.002$). In the review, no optimal dose, treatment duration, or subset of patients was identified as being more responsive to adjuvant interferon therapy. More recently, the Melanoma Disease Site Group in Canada published an analysis of high-dose IFNa regimens and found a mean relapse free survival hazard ratio of 0.76 (95% confidence interval 0.67, 0.87) and mean overall survival hazard ratio of 0.87 (95% confidence interval 0.75, 1.01) which just failed to reach statistical significance [82]. Taken together these data suggest a risk reduction for relapse of around 25% and for death of about 10% associated with high-dose IFNa. However, the usefulness of this data is further compromised by the fact that these studies took place in the era before routine sentinel lymph node staging and therefore do not provide any information on patients with currently defined N1 (Stage IIIA) melanoma, the most commonly identified high risk population in the current era.

Efforts to improve upon the therapeutic index for high-dose IFNa have focused on the use of longer acting IFN compounds, such as Pegylated IFNa, and shorter duration treatment regimens. Pegylated IFNa has been used to treat hepatitis B or C, and EORTC 18991 investigated its use in patients with resected stage III melanoma in a randomized phase III trial compared to observation [83]. Pegylated IFNa was administered subcutaneously at a dose 6 μg/kg once a week for 8 weeks followed by 3 μg/kg for 5 years. Although there was no difference in overall survival or distant metastases-free survival (DMFS), pegylated IFNa improved recurrence free survival, which led to the approval of this agent for adjuvant treatment of stage III melanoma in the US in 2011. This benefit was particularly apparent in the subset of patients with microscopic involvement of 1 lymph node and ulcerated primaries. These retrospective subset analyses, however, have yet to have independent or prospective validation.

Two studies have looked at shorter duration regimens. A study conducted in Greece examined the use of a regimen in which patients with resected high-risk melanoma were randomized to receive either a year of high-dose IFNa or a truncated regimen in which IFNa was given for only the 4-week induction period [84]. At a median follow-up of 63 months (95% CI 58.1—67.7), the median relapse free and overall survival were essentially equivalent between the two arms while patients in the 12-month treatment arm had more grade 1 to 2 hepatotoxicity, nausea/vomiting, alopecia, and neurologic toxicity. This study, while provocative, was felt to be

too small to confirm equivalence. To further investigate the utility of this shortened regimen, E1697 compared 4-week high-dose IFNa induction only with observation in 1150 patients with resected intermediate- and high-risk melanoma [85]. The median relapse-free survival was 7.3 years (95% CI 5.3, 9.8) in the observation arm and 6.8 years (95% CI 5.1, 9.0) for IFNa, while the 5-year overall survival rate was 85% (95% CI 81, 89) for observation and 82% (95% CI 78, 86) for IFNa. Because of the lack of any apparent treatment benefit, this trial was terminated early. These data call into question the value of abbreviated and modified IFN regimens and leave the original HD IFNa regimen as the, albeit controversial, standard of care for adjuvant treatment of patients with intermediate or high risk melanoma.

Biochemotherapy As another attempt to improve adjuvant treatment for high risk melanoma, patients with stage IIIB and IIIC melanoma were randomized to receive either a combination of biologics (IFNa, interleukin-2) and chemotherapy (cisplatin, vinblastine, DTIC), so called biochemotherapy, over a 9 week period or standard high-dose IFNa in an intergroup phase III study organized by the SWOG [86]. This study showed a significant improvement in relapse-free survival for the biochemotherapy arm but no improvement in overall survival. Considering the added toxicity and expense associated with the intensive inpatient biochemotherapy regimen and the lack of impact on overall survival, it is unlikely that this regimen will see much clinical application.

Other Regimens Ipilimumab is a CTLA-4 blocking monoclonal antibody which demonstrated improvement in overall survival compared to vaccine as well as chemotherapy for patients with unresectable or metastatic melanoma [87, 88]. Two large-scale Phase III trials are underway examining the value of adjuvant ipilimumab therapy, EORTC 18071 trial comparing adjuvant ipilimumab to placebo and E1609 is comparing two different doses of ipilimumab to high-dose IFNa. Accrual to these trials is now complete and results are eagerly anticipated.

1.5 Evolution of Systemic Treatment Approaches

The prognosis for patients with Stage IV melanoma has historically been poor with median survival less than a year and a 5-year overall survival rate of less than 10%. Two US Food and Drug Administration (FDA) approved drugs had been used for the treatment of patients with Stage IV melanoma in the US prior to 2011, namely, DTIC and recombinant human interleukin-2 (IL-2). Recent advances in melanoma therapy have been dramatic with the approval of ipilimumab and vemurafenib in the US in 2011 followed by approval of dabrafenib and trametinib in 2013. Greater understanding of melanoma biology coupled with the successful development of novel treatments such as anti-PD-1 antibody and new combination regimens will further improve patient outcomes in the future.

Cytotoxic chemotherapy The objective response rate of DTIC is approximately 10–20% with most responses ranging from 3 to 6 months, although long-term remis-

sions can occur in a small number of patients who achieve a complete response. Despite its FDA approval DTIC has never been shown to improve median progression free survival or overall survival compared to a control arm in any prospective randomized study. Although combinations of cytotoxic agents, including those containing DTIC or regimens adding either IFN or tamoxifen to DTIC have often produced higher response rates than DTIC alone, they also increased the toxicity without a significant improvement in survival compared to DTIC alone [89].

1.6 Immunotherapy

Interleukin-2 based therapy High-dose bolus interleukin 2 (HD IL-2) received FDA approval in 1998 for the treatment of patients with metastatic melanoma largely based on its ability to produce durable complete responses in 5–10% of patients. In a retrospective review of 270 patients treated on multiple Phase II studies, the objective response rate was 16%, with a median duration of 9 months (range 4 to 106+ months). Despite the low objective response rate, 59% of complete responders remained progression-free at 7 years and no patient responding for longer than 30 months had progressed, suggesting that some patients are "cured" [90]. Treatment, however, was associated with significant toxicity limiting its application to a select group of patients treated in specialized centers.

Efforts to improve upon the activity of IL-2 in patients with melanoma have included combinations with chemotherapy (biochemotherapy), vaccines and adoptive T cell therapy. Although several phase II trials, a small phase III trial and two meta-analyses suggested that combinations of IL-2 and cisplatin-based biochemotherapy offered benefit relative to either chemotherapy or IL-2 alone, several multi-institutional phase III trials have failed to confirm this benefit [91, 92].

Another approach to improving the activity of HD IL-2 involved the addition of a gp100 peptide vaccine. A phase III trial randomly assigned 185 patients with metastatic melanoma to HD IL-2 given alone every 3 weeks or in combination with a gp100 peptide vaccine [93]. Because of the restriction properties of the vaccine, enrollment was limited to patients who were shown to be HLA type A201. The study reported an objective response rate of 16% for the combination compared with 6% for HD IL-2 alone. There were eight complete responses (9%) in the combination arm, but only one (1%) among those treated with IL-2 alone. There was a trend toward increased overall survival (median 17.8 versus 11.1 months, $p = 0.06$), although the trial was not adequately powered to assess this endpoint. The clinical significance of this finding is uncertain considering the relatively poor response rate in patients treated with HD IL-2 alone, the current lack of availability of the specific formulation of vaccine adjuvant used in this trial and the observations that this same vaccine did not improve the efficacy of ipilimumab in a phase III trial [94] (see below).

Others have explored the efficacy of HD IL-2 in combination with adoptive transfer of tumor derived tumor reactive T cells. These approaches have included preparative regimens involving myeloablative chemotherapy with or without total body irradiation (TBI) in order to delete host immune cells and promote engraftment of adoptively transferred tumor-reactive T cells [95]. Autologous hematopoietic progenitor cell support was used in patients who received TBI. The NCI Surgery Branch recently reported the combined results from 3 separate trials. There were 52 objective responses in 93 patients (56 % response rate), including 20 (22 %) complete responses. Complete responses were ongoing at 37–82 months in 19 of the 20 responders, and the three- and 5-year actuarial survival rates for patients achieving a complete response were 100 and 93 %, respectively. Efforts to confirm these results at other centers as well as to develop a more practical treatment regimen are currently underway.

Ipilimumab The CTLA-4 receptor on T lymphocytes is a negative regulator of T cell activation that blocks positive stimulatory effects to these cells mediated through their co-stimulatory and antigen specific T cell receptors. The monoclonal antibodies ipilimumab and tremelimumab bind to CTLA-4 and thus prevent this feedback inhibition. Both have been studied in patients with melanoma, with the most extensive data and promising results being observed with ipilimumab.

Ipilimumab was studied in a placebo-controlled phase III trial in which 676 patients with previously treated advanced melanoma were randomly assigned in a 3:1:1 ratio to ipilimumab plus gp100 peptide vaccine, ipilimumab alone or gp100 vaccine alone [94]. Ipilimumab (3 mg/kg) and/or vaccine were given every 3 weeks for four doses. Patients with confirmed partial or complete response or stable disease for 3 months or more after completion of the 12 week induction period were allowed to receive re-induction with their original treatment if they subsequently had disease progression.

In this study, overall survival was significantly increased in the two groups that received ipilimumab (median 10.0 and 10.1 versus 6.4 months, in the ipilimumab plus gp100, ipilimumab alone, and gp100 groups, hazard ratios for death 0.68 and 0.66 versus gp100 alone, respectively). Treatment benefits appeared to be independent of gender, age ($\leq$65 or >65 years), stage at presentation (M0, M1a, and M1b versus M1c), baseline LDH or prior use of IL-2. Tumor response rate was also significantly improved in both groups of patients treated with ipilimumab compared to gp100 alone (5.7 and 10.9 versus 1.5 %, respectively). Further objective partial or complete responses were maintained for at least 2 years in 4 of 23 (17 %) patients treated with ipilimumab plus gp100 and 9 of 15 (60 %) with ipilimumab alone. Among 31 patients who initially received ipilimumab either alone or with gp100 and then underwent reinduction therapy with ipilimumab, six (21 %) had an objective response to retreatment, and 15 (48 %) had stable disease. Although this phase III trial limited enrollment to patients who were HLA-A*0201 positive, a retrospective analysis of four phase II trials involving ipilimumab alone showed similar activity regardless of HLA type [96]. Although patients on this trial did not have tumor profiling for BRAF mutations, recent data suggest that the activity of ipilimumab is independent of BRAF mutational status [97]. As a consequence of

this study, ipilimumab was approved for the treatment of all patients with advanced melanoma.

Ipilimumab's presumed mechanism of action is to break down tolerance to tumor-associated antigens in the melanoma. At the same time, this break down of tolerance may result in autoimmune reactions against self antigens. A wide range of immune-mediated adverse events have been observed. The most common serious manifestations include enterocolitis, hepatitis, dermatitis, and endocrinopathies. In this trial using a 3 mg/kg dose of ipilimumab immune-related adverse events occurred in approximately 60% of patients treated with ipilimumab. Grade 3 or 4 toxicity was seen in 10–15% of ipilimumab-treated patients, compared to 3% of those receiving only gp100. These side effects were typically not seen until 6 or more weeks into therapy. A somewhat higher incidence of side effects was observed with a dose of 10 mg/kg every 3 weeks in the randomized phase II trial that assessed the effects of dose on activity and toxicity [98]. Several investigators have suggested that the development of immune related toxicities correlated with benefit from therapy; however, other studies have not confirmed this correlation.

Although patients with untreated brain metastases were excluded from the phase III trial, other studies have observed antitumor activity with ipilimumab treatment in patients with brain metastases [99]. Finally, data from phase II trials suggested that a number of patients (up to 10% of those treated) exhibited apparent disease progression after 12 weeks of ipilimumab (with either larger lesions or new lesions), followed by subsequent disease regression. The overall survival outcome of these patients was similar to those exhibiting a tumor response. This led to the establishment of Immune-related Response Criteria that endeavored to capture these patients in the subset of patients achieving treatment benefit [100].

A second phase III trial involved previously untreated patients who were randomly assigned to dacarbazine plus either ipilimumab or placebo [101]. In this study, overall survival was significantly increased in patients assigned to the dacarbazine plus ipilimumab arm (median 11.2 versus 9.1 months). The overall incidence of grade 3 or 4 toxicity was significantly higher with dacarbazine plus ipilimumab (56 versus 28%). In particular, hepatic toxicity was significantly more common with the combination than with dacarbazine alone or than that previously or subsequently observed with ipilimumab alone. The increase in hepatic toxicity relative to single agent ipilimumab may be due to the fact that dacarbazine is also known to be hepatotoxic. On other hand, the incidence of other immune related toxicities (colitis, rash, hypophysitis) was less than that seen in prior studies with ipilimumab alone, perhaps suggesting that dacarbazine may have blunted and/or the higher incidence of hepatotoxicity may have pre-empted the immune toxicity profile of ipilimumab. Whether this blunting of immune toxicity by dacarbazine might have also blunted the antitumor effect of ipilimumab is a matter of speculation. However, the overall pattern of toxicity and efficacy on this trial do not support the addition of dacarbazine to ipilimumab. The relative value of the use of ipilimumab at the 10 mg/kg dose used this study and in multiple phase II studies vs. the already approved 3 mg/kg dose awaits the completion of an ongoing Phase III trial directly comparing the two doses.

A recent report of long-term survival of patients receiving ipilimumab suggests that death rate for patients followed for more than 3 years declines dramatically and that 20–25 % of patients will achieve long term survival [102].

Anti-PD1 based therapy Another immune checkpoint, programmed death 1 (PD-1), acts as an inhibitory receptor of T cells similar to CTLA-4. However, in contrast to CTLA4, the ligand for PD-1 (PDL1) appears to be expressed almost exclusively at sites of inflammation, such as in the tumor microenvironment. This observation has raised the hope that blockade of PD1 binding with PDL1 might lead to more selective restoration of immunity within the tumor microenvironment and, therefore, less associated toxicity than seen with CTLA4 blockade. Early clinical trials investigating antibodies to PD-1 and PDL1 in patients with melanoma have shown response rates ranging from 25–50 % [103, 104]. In addition a study evaluating the concurrent administration of the combination of ipilimumab and the PD1 antibody nivolumab produced rapid and deep tumor responses in patients with metastatic melanoma and an overall response rate of 53 % in a small number of patients (103). The promising results seen with various anti-PD1 and PDL1 antibodies either alone or in combination with ipilimumab have led to multiple randomized clinical trials of comparing anti-PD-1 antibodies alone or in combination with ipilimumab to standard of care in patients metastatic melanoma. In addition, efforts are underway to study the optimal coordination of immunotherapy with molecularly targeted therapies in patients with BRAF mutant melanomas.

Treatment Selection options Considerable effort has focused on identifying patients who respond to immunotherapy in the hope or restricting such treatment to those most likely to benefit. IL-2 response has been shown to be more likely in patients with normal serum LDH, or low plasma VEGF and fibronectin levels [105]. In addition, response appears to be more frequent in patients whose tumors contain mutations in BRAF or NRAS, or possess an inflammatory gene expression signature [106]. More recent studies have suggested that response to IL-2 is associated with enhancement of a pre-existing gene expression pattern within the tumor associated with immune-mediated tissue-specific destruction under the control of IFNgamma [107]. Benefit from vaccination has also been linked to tumors expressing an IFN driven chemokine signature (107). Preliminary results suggest that both PD1 antibody responsiveness and IL-2 responsive in patients with RCC may be correlated with tumor cell surface expression of PDL1 (102, 108). Furthermore, research suggests that tumor PDL1 expression is not constitutive, but is related to the secretion of IFNgamma by of tumor reactive CD8 T cells in the microenvironment. Thus, effective immunotherapy may require pre-existence of tumor specific immunity within the microenvironment and the use of agents that can either drive T cell function (HD IL-2 or vaccines) or block inherent immunoregulatory signals (ipilimumab, or anti-PD1). Several current studies are underway to validate these predictive biomarkers for specific immunotherapies as well as to determine if combinations of immunotherapy with either other immunotherapies or molecularly targeted agents could convert non-immune responsive tumors into those capable of responding.

1.7 Conclusion

The diagnosis and treatment of patients with all stages of melanoma has continued to evolve over the course of the past century. Although until recently the most effective treatment approaches have been surgical, the greater understanding of the tumor microenvironment have led to advances in immune based systemic treatment options for patients with metastatic melanoma. The challenge now is to determine how best to use these agents alone, in sequence and in combination, how to predict patients destined to respond to therapies and determine timing and mechanisms of resistance and how to move these approaches into the adjuvant settings. In addition, considerable investigation is needed to determine how best to integrate these novel immune based therapies with the rapidly expanding knowledge of molecular changes within the tumor cells themselves and the treatment approaches being developed to target these oncogenic drivers that are described in this book.

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