Tailoring the Treatment of Melanoma: Implications for Personalized Medicine

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MELANOMA TREATMENT: A BRIEF HISTORY

Evidence of melanoma emerged early in the development of human civilization. Pre-Columbian mummies carbon-dated to approximately 2,400 years ago show signs of metastatic melanoma [3]. In the 5th century BCE, Hippocrates first textually described melanoma [4]. Records from after this time are scattered; the physician Rufus of Ephesus mentioned the disease in the 1st century BCE, and several texts from 17th- and 18th-century Europe describe “fatal black tumors” with widespread metastases [3]. In 1804, Rene Laennec identified metastatic melanoma in coal miners’ lungs [5]. In 1820, William Norris, a British general practitioner, followed a melanoma patient for 3 years and observed the tumor’s widespread metastasis.

Until the mid-20th century, surgery was the sole treatment available; the standard treatment for melanoma was wide resection and removal of adjacent lymph nodes [4,6]. In 1966, Wallace Clark characterized melanoma into five levels by their depth of invasion, with Level I defined as confinement to the epidermis and Level V as invasion into subcutaneous fat [7]. Subsequent researchers noted that survival rate inversely correlated with depth of invasion, with Level V carrying the worst prognosis [8]. The current American Joint Committee on Cancer (AJCC†) staging system for melanoma includes tumor depth, lymph node infiltration, and metastases among its criteria (Table 1), and studies show that the most important prognostic factors are tumor depth, the presence of ulceration, and lymph node involvement [9].
Table 1. T, N, and M staging scheme for melanoma.

| Primary Tumor | Thickness (mm) | Sub-classification |
|---------------|----------------|--------------------|
| Tis           | N/A            | N/A                |
| T1            | ≤ 1.00         | a: W/o ulceration AND mitosis < 1/mm²  
|               |                | b: W/ ulceration OR mitoses ≥ 1/mm² |
| T2            | 1.01-2.00      | a: W/o ulceration  
|               |                | b: W/ ulceration   |
| T3            | 2.01-4.00      | a: W/o ulceration  
|               |                | b: W/ ulceration   |
| T4            | > 4.00         | a: W/o ulceration  
|               |                | b: W/ ulceration   |

| Regional Lymph Node Involvement |
|---------------------------------|
| N Stage | # of Nodes | Sub-classification |
|---------|------------|--------------------|
| N0      | 0          | N/A                |
| N1      | 1          | a: micrometastasis  
|         |            | b: macrometastases |
| N2      | 2-3        | a: micrometastasis  
|         |            | b: macrometastases |
|         |            | c: in-transit metastases WITHOUT metastatic nodes |
| N3      | 4+, or matted nodes, or in-transit metastases with metastatic node(s) |

| Distant Metastasis |
|--------------------|
| M Stage | Site | Sub-classification |
|---------|------|--------------------|
| M0      | No detectable distant mets | N/A |
| M1a     | Distant skin, subcutaneous, or nodal mets | LDH Normal |
| M1b     | Lung mets | LDH Normal |
| M1c     | All other visceral mets | LDH Normal |
|         | Any distant mets | LDH Elevated |

Micrometastases are found by sentinel node biopsy, while macrometastases are clinically detectable and confirmed by pathology. LDH = Lactate Dehydrogenase.

showed a 40 percent to 50 percent response rate in a Phase III trial in Stage IV metastatic melanoma patients [13].

**RECENT DEVELOPMENTS IN MELANOMA TREATMENT**

**Magic Bullets**

Chemotherapeutics like melphalan represent some of the first steps toward the modern paradigm of personalized medicine. Paul Ehrlich’s idea of the “magic bullet” — a drug that targets diseased cells while leaving healthy tissue intact — guides this paradigm. Ideally, anti-neoplastic drugs would preferentially target rapidly proliferating and genomically unstable cancer cells over non-transformed healthy cells; however, almost all early chemotherapeutics target proteins and structures present in both normal and cancerous cells, leading to dose-limiting systemic side effects [14].

A sea change in cancer treatment occurred in 2001 with the FDA’s approval of imatinib mesylate, a targeted
therapy for chronic myelogenous leukemia (CML). Approximately 90 percent of CML cases show a specific 9- to-22 translocation called the Philadelphia chromosome, which encodes the oncogenic BCR-ABL fusion protein [15]. As the first anticancer drug developed through rational drug design, imatinib was tailored to target BCR-ABL [16]. Blockbuster results ensued; for chronic phase CML patients, 8-year survival rates increased from 15 percent in 1975 to 87 percent after 2001 [17-18]. While imatinib may not be perfectly “magic” (since it can also target other wild-type kinases like c-KIT [19] and PDGFR-R [20] and is not free of side effects [21]), its success ushered in a new age of personalized cancer treatment [22].

This “magic bullet” principle also has been applied to melanoma treatment. Once research began focusing on the genetic insults necessary for melanoma pathogenesis, a panoply of targets became available. Studies of melanoma cell lines and excised tumors revealed activating mutations in NRAS, a member of the Ras superfamily of monomeric G-protein oncogenes [23-24]. Subsequent studies have shown that activating mutations in the MAP kinase (MAPK) signaling pathway are often found in melanoma [25-26]. In 2002, researchers determined that mutations in NRAS or its downstream target BRAF, a serine-threonine kinase, are present in more than half of metastatic melanoma cases [27-28]. Of those BRAF mutations, all were in the kinase domain, and about 80 percent harbored a specific valine to glutamic acid substitution (V600E) [25].

**BRAF as a Therapeutic Focus**

Because it is mutated in a multitude of cancers, Ras presents an attractive therapeutic target [29]. Unfortunately, in spite of 30 years of attempts since its discovery, the Ras superfamily remains an elusive drug target [30]. While some early experimental Ras inhibitors did cause tumor regression in melanoma cell line models [31], those drugs could not pass phase II and III trials [32-33]. Intriguingly, a class of cysteine-reactive inhibitors that bind to a newly discovered allosteric pocket of Ras was recently tested and showed promising, if preliminary, results [34-35].

On the other hand, however, the discovery of BRAFV600E has proven to be a turning point for melanoma treatment. Although initial attempts using the non-selective BRAF inhibitor sorafenib were not successful [36], a breakthrough occurred in 2008 with an *in vitro* study that showed that the small molecule PLX-4032 could selectively target BRAFV600E mutant cell lines in culture [37-39]. PLX-4032 was designed through a particularly innovative process called “fragment-based lead discovery.” Because the chemical backbones for previously discovered kinase inhibitors did not show affinity for BRAF, researchers screened a library of ~20,000 diverse scaffolds at high concentration against a panel of five kinases, co-crystallized compounds that would inhibit at least three of the five to determine their conformation when bound to the target, and rationally optimized those lead compounds against the structure of BRAFV600E, thus resulting in a compound with very high specificity [40]. The ability to develop compounds selective not only for a given kinase, but also for a mutant of that kinase, demonstrates the potential of personalized medicine.

After PLX-4032’s discovery and target confirmation, a phase I trial showed great promise, inducing tumor regression in 81 percent of patients [41]. In 2011, a phase III trial demonstrated a 63 percent relative reduction in risk of death, leading to FDA approval of PLX-4032 (now called vemurafenib) that year [42]. Subsequent trials confirmed that vemurafenib increased median progression-free survival in metastatic melanoma to 5.3 months, compared to 1.6 months for dacarbazine [43]. Since its approval, vemurafenib has become a mainstay for treatment of unresectable malignant melanoma.

However, vemurafenib is not a panacea. It was soon discovered that after a phase of rapid tumor regression, patients would often relapse after 6 to 8 months of treatment [44]. Furthermore, vemurafenib can activate BRAF in wild-type cells, inducing squamous cell carcinomas and keratoacanthomas from cells that had previously harbored non-pathogenic Ras mutations [45].

There are several mechanisms for this escape phenomenon. For instance, neoplastic cells can acquire further activating mutations in NRAS, receptor tyrosine kinases, or other members of the MAPK pathway [46-47]. In another study, cells expressing BRAFV600E treated with vemurafenib began to express a new 61kDa splice variant that dimerizes and activates even in the presence of inhibitor [48], leading to paradoxical activation of the MAPK pathway. Finally, in wild-type cells, vemurafenib can stabilize the formation of active BRAF dimers (including BRAF homodimers and heterodimers with homolog CRAF), increasing MAPK signaling in a Ras-dependent manner [49-50].

Subsequent to these observations, the strategy of blocking multiple members of the MAPK pathway was advanced. In a phase III clinical trial published in 2015, a combined regimen of a second-generation BRAF inhibitor (dabrafenib) and a small molecule inhibitor of the BRAF target MEK (trametinib) increased median progression-free lifespan to 11.4 months, compared to 7.3 months for vemurafenib monotherapy [51]. This result was consistent with other trials that compared combination therapy to BRAF inhibition alone [52-54]. In addition, combination therapies showed a decreased incidence of other skin cancers compared to monotherapy without increased overall toxicity [52]. It remains to be seen, however, what further adaptations melanoma cells will acquire in response to combination therapy [55]. While a recent study does show that melanoma cell lines can overcome combined BRAF-MEK inhibition by amplifying BRAF to supraphysiological levels, this mechanism has not yet been confirmed in patients [56].

**An Alternative: Personalized Immunotherapy**

One of the many checks against carcinogenesis is tumor surveillance by the immune system. In addition to attacking
oncogenic viruses and pathogens that promote a tumorigenic inflammatory state, innate immune cells like natural killer cells and T lymphocytes directly target transformed cells, eliminating subclinical tumors before they can spread [57]. However, as tumorigenesis continues, this equilibrium between the immune system and potential tumors begins to shift in the tumor’s favor. Over time, in a process called “immunoediting,” the immune system selects for less immunogenic tumors that are able to escape surveillance [58].

While cancer immunoediting was a controversial scientific hypothesis until the 21st century, immunotherapy for melanoma has been employed in the clinic since the 1990s. In 1992, the FDA approved high-dose interleukin-2 (IL-2) for patients with metastatic melanoma, and it remained the preferred treatment until the introduction of BRAF inhibitors [59]. In one study, high-dose IL-2 achieved a 16 percent response rate in patients with metastatic melanoma [60] and could even induce complete regression of the tumor in a small subset of patients [61-62]. While the exact mechanisms of IL-2’s antitumor effect are not fully explicated, it is dependent on T-cell help and can induce the action of tumor-specific T-cells [63-64].

Melanoma is an especially immunogenic cancer. Several melanoma-specific antigens have been identified, and patients often have circulating CD8+ T-cells specific for melanoma antigens, although they show blunted responses and signs of exhaustion, possibly due to the action of regulatory T-cells (Tregs) [65-68]. These observations drove the successful development of immunostimulators that allow anti-tumor T-cells to bypass Treg-mediated checkpoints. Two such drugs are ipilimumab, a monoclonal antibody that blocks the immune checkpoint receptor CTLA-4 on T-cells, and nivolumab, a monoclonal antibody directed against checkpoint receptor PD-1 expressed on T-cells, B-cells, and NK-cells [69]. Trials demonstrate that ipilimumab and nivolumab are effective monotherapies in patients with wild-type BRAF [70-71]. In addition, a new PD-1 inhibitor called pembrolizumab was approved by the FDA in 2014 and showed almost double the progression-free survival rate at 6 months as ipilimumab in a phase III trial [72].

Several attempts have been made to combine immunostimulators with other treatments, though concerns over toxicity may limit their application. A phase I dose-escalation trial showed that ipilimumab and nivolumab combined significantly increases the percentage of objective responses and complete responses over ipilimumab monotherapy, though at the cost of double the rate of grade 3 or 4 adverse events in the combination arm [73]. In fact, a third of the patients in the combination arm discontinued the treatment due to toxic effects, raising concerns from clinicians [74]. Combining immunostimulators with MAPK pathway inhibitors has the potential to improve treatment even further [75], though a 2013 phase I trial combining vemurafenib and ipilimumab was terminated due to hepatotoxicity [76]. More recently, a 2015 study in a mouse model showed that a triple therapy of BRAF inhibition, MEK inhibition, and either adoptive T-cell transfer or PD-1 inhibition was able to induce durable tumor responses, but it has not yet been tested in humans [77].

Such combinations are not limited to pharmacologic therapies. A 2013 retrospective study showed that the combination of ipilimumab and radiotherapy (stereotactic radiosurgery or whole-brain radiation) had superior response rates to radiation alone for treating brain metastases [78]. While some data suggests that ipilimumab and radiation may have a synergistic effect, more prospective studies and elucidation of side effects are needed [79-80].

**CONCLUSION: TRUE PERSONALIZATION ON THE HORIZON?**

As with many cancers, personalized treatment of melanoma is still in its infancy. Current treatment guidelines for metastatic melanoma recommend testing metastatic tissue for relevant mutations (NRAS, BRAF, KIT, GNAQ/11, and/or BAP1) in order to determine prognosis and to personalize treatment regimens [81-82]. High-throughput sequencing of tumor samples has revealed more genes of therapeutic interest, with some studies suggesting larger panels of mutations for finer personalization [83-86]. In a landmark study characterizing 331 melanomas at the DNA, RNA, and protein level, the Cancer Genome Atlas Network suggested a genomic classification of tumors by their BRAF, NRAS, and NF1 status and revealed the existence of several other mutations and subtypes of the disease [87].

On the immunological side, two recent advances in personalized cancer medicine are particularly noteworthy. First, in a 2015 study, three stage III melanoma patients’ tumors were exome sequenced and used to create a peptide vaccine tailored to the neo-antigens in each tumor; these vaccines managed to induce a diverse T-cell response to tumor-specific antigens in all three patients [88]. While the authors did not report a clinical response to their vaccines, the fact that such an immune response can be elicited holds promise for future personalized immunotherapy.

A second relevant development is adoptive T-cell therapy (or chimeric antigen receptor T-cell therapy), in which autologous T-cells are engineered to directly target tumor antigen. Researchers extract T-cells from a patient, genetically modify them to express an artificial T-cell receptor engineered against the target antigen, and reinfuse the transformed cells [89]. Adoptive T-cell therapy has been successfully used to treat leukemia [90-91], and adoptive transfer experiments in a mouse xenograft model of human melanoma have demonstrated tumor regression, though human trials have not yet been conducted [92-93].

In summary, melanoma therapy has become increasingly selective, from narrower and narrower surgical margins to small molecules that bind to specific oncoproteins. Targeted therapies have greatly improved outcomes, and more molecular targets are under consideration. In the future, the ability to narrowly tailor cancer treatment-based
on panels of hundreds of mutations and neo-antigens, perhaps even using the patient’s own immune system to attack the tumor, has the potential to usher in an era of highly selective tumor targeting and ever-increasing cure rates.

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