Dermatologists are adept at treating and diagnosing melanoma. However, diagnosing and treating melanomas is a vastly changing topic as newer medications and diagnostic techniques become available. It is important for practitioners to be aware of newer adjunctive methods to help monitor, treat, and assess patients as the ability to diagnose and treat melanoma advances and increases. As the primary physician a patient seeks, dermatologists must be aware of new drugs to treat metastatic melanoma in an effort to counsel, reassure, and treat their patients.

**IMAGING AND DIAGNOSTIC TECHNIQUES**

Imaging techniques such as the use of dermatoscopy, imaging booths, multispectral imaging, confocal microscopy and electrical impedance spectroscopy may be adjuncts in making the diagnosis of early lesions enabling early treatment when success is excellent. The goal of these adjunctive tools is to obtain reproducible, high-resolution, *in vivo* imaging that can be used immediately with the patient that has the same or greater diagnostic accuracy as a biopsy.

Electrical impedance spectroscopy is being investigated as such a diagnostic tool. A tiny electrical impulse would travel from one probe to another through the skin. Rather than using light-based stimulation, this method uses a different physical property of cells. Since malignant and benign cells impede currents differently, they can be divided in this manner to differentiate benign from malignant lesions. A 95 percent sensitivity and 49 percent specificity for malignant cells using this technique was reported in a multicenter study in 2011.

Long-wave infrared technology is also being used to screen for noninvasive and malignant lesions. This device uses thermal signatures of lesions pre-treated with a temperature stimulus. A pilot study showed a 96.97 percent sensitivity and 78.05 percent specificity. Biopsy is still the mainstay for diagnosis with nearly 100 percent specificity. Similarly, DNA from ultraviolet induced cells is different in cancerous cells like those in melanoma and is being studied as an early marker and for diagnostic purposes.

Cells from lesions may be harvested noninvasively. Derm Tech International’s Pigmented Lesion assay was recently submitted for approval; it involves the application of an adhesive patch to collect mRNA from pigmented lesions. This is evaluated by a technique called mRNA expression profiling, which looks at patterns of the mRNA expression and divides the lesion into benign
or malignant.4 This could allow the clinician to decide exactly what lesion needs a biopsy. It would be especially useful in patients with borderline lesions such as in those with multiple dysplastic nevus syndrome or those with benign lesions that mimic melanoma under the microscope like deep penetrating nevi, dysplastic nevi, and Spitz nevi. The technique may be useful in therapeutics as well by finding specific mutations in the melanomas, which would allow for the use of targeted therapies for treatment. It could also predict which melanomas would be more aggressive. This is of prognostic importance and would not replace the biopsy diagnosis.4,5

Another assay, the DecisionDx-Melanoma test, is an mRNA expression profile test that can examine previously excised melanomas. It can give information to classify melanomas into groups of how aggressive they are. Could this allow for increased surveillance and treatment of melanomas that are genetically proven to become more aggressive? This gene expression profiling (GEP), available since mid-2013, was compared to sentinel node biopsy, the currently used prognostic test with excellent results. The positive predictive values were comparable; however, the negative predictive value (accuracy of negative results) was reportedly better with GEP, 79 versus 59 percent. Thus, GEP may identify patients who have a high risk despite a negative SLNB.5 It could be a useful adjunctive test for patients who decline a surgical (SLNB) approach as well.

FISH probes, fluorescent in situ hybridization, are used to evaluate DNA for copy number changes and evaluate borderline histopathologic lesions. By looking at patterns of DNA, the lesion can be divided into benign or malignant and addressed accordingly. Myriad Genetics uses mRNA expression in a similar way.4,5

A murine monoclonal antibody, VE1 anti-BRAF V600E, staining has been studied and shown to have a high sensitivity and specificity for melanomas with the V600E BRAF mutation. It has been used in colon and thyroid cancer as well as hair cell leukemia. This study could be helpful as an adjunct to DNA analysis identifying V600E status in patients who have insufficient tissue for BRAF analysis.4,5

These tools can enhance diagnosis and prognostic evaluations, but cannot replace the acute expertise of the board certified dermatologist. There can be false negatives and false positives requiring the clinician’s experience and evaluation.

**TREATING ADVANCED MELANOMA**

Early melanomas are easily excised by dermatologists and have an excellent prognosis. This has not been the case for metastatic melanoma, however, new combination treatments are on the horizon, and there are many options. Interferon has been used, but without much success. The latest advances in therapy rely upon two modes of attack. Immunotherapy targets the host and targeted therapy attacks tumors directly. Melanoma is a mix of subtypes of specific mutations. BRAF kinase mediates cell proliferation and 50 percent of these mutations are BRAF positive; therefore, inhibitors like ipilimumab and vemurafenib were great advances. Results, however, were not long lasting.

Dabrafenib, an oral BRAF mutation inhibitor, was approved in 2013. Patients responded initially, but tumors recurred. Trametinib, an oral drug that targets mitogen-activated protein kinase (MAPK) kinase (MEK) is being used in combination with dabrafenib in an effort to achieve more longer lasting remissions.6 Iplimunab, the first drug approved for metastatic melanoma in 2011, is infused every three weeks for 12 weeks and extends life about three months. It is being combined with nivolumab, a new drug that shrinks or stabilizes the lesions as a PD-1 inhibitor. PD-1 is expressed by activated T cells and is involved with chronic inflammation. Half of the patients treated showed an 80 percent reduction in lesions for one year and 79 percent survived for 2 years. Combination therapy seems to hold back resistance and nivolumab was FDA approved in December and is now available.9 The size of the tumors and pretreatment is an indicator of the expected response to MK3475 (pembrolizumab) was approved by the FDA in September 2014, but will still require monitoring for evidence of improved survival. This drug targets programmed death pathway (PD-1). There was a 69 percent survival of one year. PD-L1 is also a protein that activates PD-1 and suppresses T cells so that they cannot attack cancer cells. Suppressing PD-1 and PD-L1 seems to be more effective than suppressing CTLA-4; however, there

“Melanoma is a mix of subtypes of specific mutations. BRAF kinase mediates cell proliferation and 50 percent of these mutations are BRAF positive; therefore, inhibitors like ipilimumab and vemurafenib were great advances. Results, however, were not long lasting.”
is another study, EORTIC, indicating the early use of ipilimumab (a CTLA-4) in stage III melanomas at a higher dosage (10 mg/kg) than the currently approved 3 mg/kg for stage IV melanomas, delays recurrences.

Along with nivolumab, lamrolizumab is expected to have a special rolling New Drug Application submission in the first half of 2015. Japan recently approved nivolumab for “unresectable melanoma”. These drugs also target programmed death 1 (PD-1), a receptor on T cells that helps shut down the immune system after assaults such as the cold virus. By blocking PD-1 directly, the regulatory mechanisms are lifted and the immune system can target cancer continuously. Melanoma uses PD-1 ligand that is expressed on cancer cells and binds to PD-1 to hide from the immune system. In one study, 45 percent of patients stayed stable at 24-weeks.

A Phase III CheckMate-066 study randomly divided 1000 patients into two groups. One received 3mg/kg intravenous nivolumab every two weeks or dacarbazine 1000 mg/m2 IV every three weeks. Overall survival was the primary endpoint for these Stage III or IV BRAF positive unresectable lesion patients. The use of PD-L1 as a predictive marker was also analyzed. Full publication of results is pending.

High dose IL-2 is being used and patients who respond in the first 18 months do well; however, the infusion must be monitored well due to reports of fatal reactions.

Studies have shown a new pathway for melanoma metastasis. Multiple exposures to ultraviolet radiation not only causes tumor-inducing DNA mutations, but causes metastasis. Neutrophils from damaged epidermal keratinocytes are recruited and activated. Angiogenesis is increased and melanoma cells migrate to endothelial cells. There is angiotropism, perivascular invasion, and dissemination of melanoma. During extravascular migratory metastasis, the cells crawl along external areas of vascular channels in a pericytic location. Besides the intravascular dissemination of tumor cells, this new pathway of metastasis could allow for the development of new targeted tumor cell migration and suppression treatments. Dr. Claire Lugassy is trying to identify new targets for angiotropic melanoma cells and endothelial cells to specifically interfere with EVMM and metastatic progression.

Melanoma is resistant to the usual doses of chemotherapeutic medications. Higher dosages incur non-tolerable side effects making them useless. Yang, et. al. treated murine melanoma weakly for three doses of 10 mg/kg cisplatin in five groups—nontreated, intravenous, IV nano hyaluronan-conjugated (HA-Pt), subcutaneous peri-tumoral, and sc peri-tumoral HA-Pt. Tumors shrank in the sc per-tumoral HA-Pt group; therefore, this may be a potential therapeutic option in certain melanomas.

Surgical excision remains the key treatment of melanoma, unique lesions, and metastasis limited to the skin without nodal involvement. If there are too many lesions for these procedures, local injections of Bacillus Calmette-Guerin (BCG), Interferon, and IL-2 may be used.

OTHER CURRENT STUDIES

Antibodies to melanoma have been of interest for some time; however, results have not proved efficacious. A peptide triple vaccine (gp 100, MART-1/Melan-A, and tyrosinase HLA-A2 epitopes) is in a trial and has shown a two-fold survival. Again, there is another approach using antibodies to immunoregulatory checkpoint molecules like CTLA-4, a protein produced by T cells that prevents autoimmunity, such as ipilimumab. This gives a broader stimulation of the immune system and blockers are being studied as an adjuvant therapy.

Combination therapies using anti-CTLA-4 and anti-PD-1 antibodies or immunotherapy targeted therapy increase the immune responses, but they may have many adverse effects. Further studies must be undertaken to find the patients for each treatment.

The two main types of drugs in clinical trials today are...
immune checkpoint inhibitors and tumor infiltrating lymphocytes (TILs) that attack T-cells.

The NIH and the Moffit Cancer Center researchers have identified possible therapeutic targets for drug-resistant melanoma using an analytic technique called liquid chromatography–multiple reaction monitoring mass spectroscopy to measure biomarkers or molecules in blood and tissue to see if cancer is present and if there is a response to treatment. Patients with higher expressions of immune genes have better outcomes. This can be used as a predictive test to see if a patient will respond well to CTLA-4.

The University of New Mexico has technology and is developing a new method to diagnose and treat metastatic melanoma using a peptide that binds to melanoma cells. The physician loads it with imaging and therapeutic radionucleotides to assess spread and target tumors for treatment.

The International Journal of Cancer reports the use of IL-12p40 to predict increase change of melanoma recurrence is stage I/II melanomas. Overall survival rate is reported as HR=1.05. No relationship is noted between IL-12p40 and stage III/IV melanoma.

Another experimental medication in a current Phase III study, cobiimetinib, extended life when used in combination with vemurafenib.

Studies examining new ways of using existing drugs to prolong life continue. Ipilimumab is approved for stage IV inoperable melanoma at 3 mg/kg for four doses. Used at a higher dose than approved carries more toxicities; however, it is the first adjuvant therapy to extend recurrence free survival in stage III melanoma. Interferon alfa 2a and pegylated interferon have been used in this manner, but with minimal success.

Other studies include BRAF + MEK, BRAF + p13K, BRAF P13K+mTOR, ECOG1609 and BRIM 8 versus placebo, ipilimumab as compared to high dose interferon, and studies considering use of these adjuvant therapies earlier in diagnosis such as in patients that are stage 2C. Usage as first line treatments in genetic mutation patients, KIT and MRAS trials are being done at the University of Pennsylvania. Used both in combination and as monotherapy, intrahumoral injection talimogene laherparevec (T-VEC), an oncolytic virus, showed promise in patients with unresectable melanoma. This is a modified herpes virus that infects tumor cells and in injected directly into the tumor. This is auto-vaccination. The virus lysis cells and release inflammatory signals that cause the immune system to attack other tumor cells. The phase III OPTiM study in stage IIIB/C and IV patients randomized the use of intralesional T-VEC or subcutaneous granulocyte macrophage-colony-stimulating factor (GM-CSF) every two weeks; T-VEC had 23.3 months overall survival and GM-CSF had 18.9 months. Safety studies with T-VEC and ipilimumab are underway as are studies combining T-VEC and PD-1 inhibitor MK-3475 which may prove to be a better combination. Other targets of immune activation include CD40, LAG-3, GITR, and OX-40.

Progress is being made, the field is expanding and literally developing daily. It is difficult to keep up with each new regime. No therapy is advanced enough to be considered state of the art with true therapeutic results necessitating ongoing studies. Combination therapies and earlier intervention with adjuvants hold much promise.

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