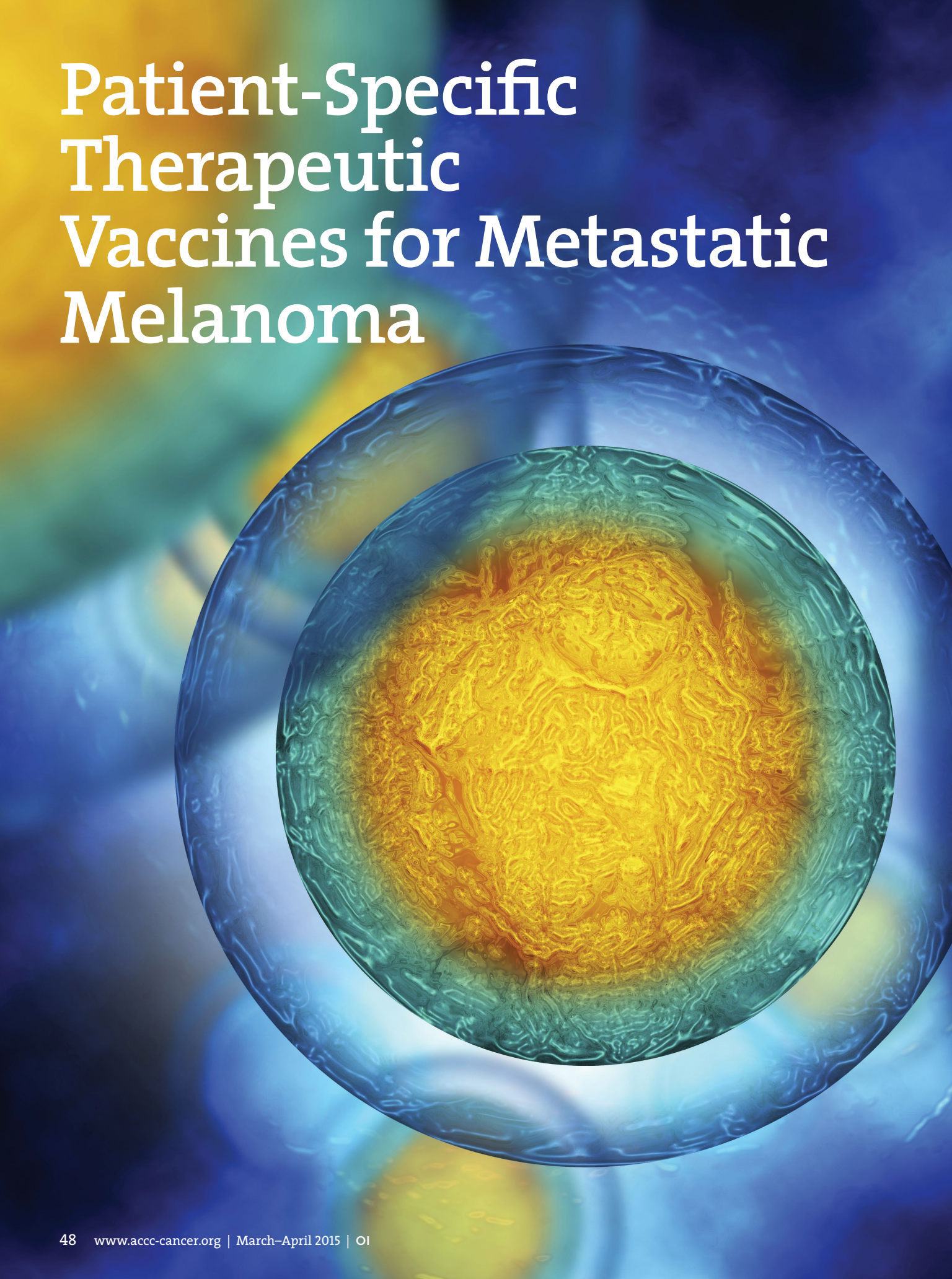
Patient-Specific Therapeutic Vaccines for Metastatic Melanoma



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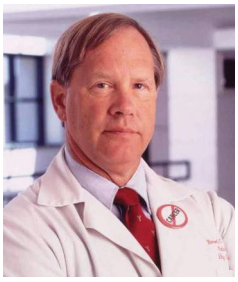


The only standard treatments for metastatic melanoma that have been associated with long-term overall survival (OS) are surgical resection, and immunotherapies that include the immune-stimulating cytokine interleukin-2 (IL2), the anti-cytotoxic T lymphocyte antigen-4 (CTLA-4) monoclonal antibody ipilimumab, and the anti-programmed death 1 (PD1) monoclonal antibodies nivolumab and pembrolizumab (aka lambrolizumab). Long-term OS has not been enhanced by classical chemotherapy, or agents that target enzymes associated with *BRAF* and MET. Until recently, 5-year OS rates for patients with unresectable metastatic melanoma were less than 10 percent.[1,2] For many years dacarbazine or temozolomide chemotherapy alone, or in combination with other chemotherapies, was the most frequently used treatment for patients with metastatic melanoma. In randomized trials, 2-year survival rates with these agents were less than 20 percent;[3-6] 5-year OS rates were not reported. Combinations of chemotherapy also failed to improve long-term survival.[3, 7-10]

Surgical Resection

The Society for Immunotherapy of Cancer (SITC) guidelines for treatment of metastatic melanoma recommend surgical resection as the treatment of choice in patients whose disease can be completely resected.[11] Surgical resection of metastatic disease is associated with 5-year OS rates of between 25 and 35 percent, depending on patient selection and the sites of metastases.[12-14] This approach is limited to patients who are fit for surgery, and typically to those who have either a single metastatic site, or a few metastases limited to a single organ that can be readily resected (e.g., lung segmentectomy, section of bowel, lymph node station, or hepatic lobe), or readily accessible solitary sites in two or three separate organs. It has been assumed that an underlying immune response makes long-term OS possible in post-metastasectomy patients, many of whom undergo repeated resections of recurrent metastases over the course of their disease. Such patients were the focus of randomized trials testing a vaccine derived from allogeneic tumor cell lines,[15] and granulocyte-macrophage colony stimulating factor (GM-CSF), and/or melanoma peptides gp100, MART-1, and tryosinase.[16] Unfortunately none of these improved survival compared to placebo-based control arms.

BRAF and MET Inhibitors

In patients whose tumors express V600 *BRAF* mutations, oral, targeted enzyme inhibitors are useful for gaining rapid control of widespread or rapidly progressing metastatic disease.[17] For aberrant epidermal growth factor signal transduction, *BRAF* inhibitors,[5,18,19] and MET inhibitors,[20] both have activity as single agents, but the combination of *BRAF* and MET inhibitors, such as dabrafanib plus tremitinib,[21] or vemurafinib plus cobimetinib,[22] is preferred. These combinations not only produce higher response rates, but actually decrease the risk of secondary cutaneous tumors. With these combinations, an objective response rate (ORR) in the range of 75 to 85 percent has been observed. Unfortunately only about 10 percent of patients exhibit complete responses, and resistance tends to develop within a few months,[23] such that median progression-free survival (PFS) is only one year. In randomized trials, these enzyme inhibitors were superior to dacarbazine or temozolomide in terms of ORR and PFS, but they had no significant impact on long-term OS. Treatments that enhance recognition of tumor associated antigens (TAA) may prolong the benefit of these agents, and it has been suggested that *BRAF* mutations are associated with increased TAA expression.[24] For these reasons, and their limited impact on long-term OS, many melanoma thought-leaders recommend immunotherapy as first-line treatment of unresectable metastatic melanoma patients, even if they have the V600E mutations.[11,25]

Interleukin-2

Interleukin-2 (or IL2) has been commercially available since 1992, but was not specifically approved for marketing as melanoma therapy until 1998, based on pooled data on 270 patients from 8 Phase II trials.[26] Although the ORR was only 16 percent, about half were complete responses that were quite durable. Various high-dose IL2 trials have confirmed 5-year OS rates of 15 percent in patients with metastatic melanoma.[27-29] Combining chemotherapy with IL2 results in higher ORR, and more toxicity, but does not prolong OS compared to sequencing of such therapies.[30,31] Unfortunately IL2 itself is quite toxic and requires hospitalization for administration and monitoring.[32] However, the side effects tend to reverse quickly once treatment is discontinued. The typical

treatment plan involves no more than two cycles of therapy over two months.[33] Most patients have stable disease rather than an objective response three months after starting treatment. IL2 works by stimulating existing immune responses to TAA via both the innate immune system (natural killer cells) and the adaptive immune system (cytotoxic T lymphocytes). Therefore, it is also a treatment that might be more effective if TAA recognition is enhanced by vaccination. In a randomized trial IL2 plus gp100 vaccine was associated with a higher response rate and longer PFS compared to IL2 alone, but 5-year OS was still only 15 percent in both arms,[29] which was similar to results for 131 melanoma patients treated in 3 Phase II trials with IL2 plus gp100.[28] In a retrospective analysis, 5-year OS rates were three times longer (39 percent vs 13 percent) in patients treated with IL2 plus an autologous vaccine than with IL2 alone.[34]

Monoclonal Antibodies

Recently there has been unprecedented success in the treatment of unresectable melanoma with monoclonal antibodies that target immune-inhibitory checkpoint molecules, such as cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death molecules (PD1) or PD ligands.[35,36] In a recent study by Hodi et al., despite a relatively low ORR, the anti-CTLA-4 antibody ipilimumab, with or without gp100 peptide vaccine, was associated with a longer OS than the control arm of gp100 in patients who had progressed despite prior immunotherapy (IL2 or interferon) or chemotherapy (dacarbazine or temozolomide).[37] Patients treated in 3 Phase II trials testing various doses of ipilimumab had a 4-year survival rate of about 20 percent from the start of treatment.[38] Ipilimumab is administered as four infusions over three months. Its major drawback is immune-related adverse events (IRAE) associated with the release of repressed autoimmune responses.[39] These IRAE include colitis, dermatitis, hepatitis, iritis, hypophysitis, pneumonitis, and nephritis. IRAE are problematic, and can be severe to life-threatening in up to one-third of patients, although they are reversible if recognized in a timely manner and treated appropriately.

More recently there has been great excitement over monoclonal antibodies that block PD1 and PDL1, which, like CTLA-4, are associated with immune suppression. In patients with metastatic melanoma, antibodies that block these checkpoint inhibitors have been associated with ORR of 25 to 35 percent,[40-43] and 2-year OS rates of more than 40 percent.[44] Similar to what was seen with ipilimumab, some patients have experienced delayed responses, or even early disease progression followed by tumor regression.[45] Long-term disease control has been documented after discontinuation of therapy. Five-year survival rates are projected to be about 30 to 40 percent. Nivolumab and pembrolizumab also cause IRAE, but the severity is usually much less than observed with ipilimumab, except possibly for pneumonitis. Response rates associated with anti-PD1 inhibitors are similar or slightly higher in patients previously treated with ipilimumab.[43] Concurrent administration of the anti-CTLA-4 ipilimumab plus the anti-PD1 nivolumab was associated with an ORR of 40 percent, but also had a 53 percent rate of severe and life-threatening IRAE.[46]

Vaccines & Checkpoint Inhibitors

The checkpoint molecules are key mediators in the suppression of anti-TAA immune responses that are part of the cancer versus immunity evolutionary battle.[35,36,47,48] CTLA-4 interferes with the interaction between antigen presenting cells and T lymphocytes, while the binding of PDL1 to PD1 causes anergy (a state of immune unresponsiveness) in T cells and other immune cells. In tumors, PDL1 is found on the surface of tumor cells, and in the extracellular space. PD1 and PDL1 are both expressed on dendritic cells. Interference with the binding of PDL1 to PD1 can be accomplished by giving antibodies that block either molecule. Metaphorically speaking, interference with these interactions effectively takes the brakes off of existing host anti-cancer immune responses that have been repressed. Unfortunately, not all patients benefit from these checkpoint inhibitor immunotherapies, and it appears that 5-year OS rates following such therapies will be less than 50 percent; so adjunctive nontoxic therapies for patients with metastatic melanoma are still needed. Because of persisting concerns regarding IRAE, it is not clear whether the anti-checkpoint agents will have a role as adjunctive therapies after metastasectomy.

When anti-checkpoint therapies are ineffective, the explanation may be the absence of recognition of TAA. One way to enhance TAA recognition is vaccination. Experiments in M16 melanoma animal models have demonstrated a benefit for adding a GM-CSF secreting vaccine with both anti-CTLA-4,[49] and anti-PD1 antibodies;[50] the combinations were superior to vaccine alone and to either anti-checkpoint antibody alone. This is why the placebo-controlled randomized trial that led to approval of ipilimumab randomized patients 3:1:1 to ipilimumab plus gp100 vaccine, gp100 vaccine alone, and ipilimumab alone because of the belief that the combination (of ipilimumab plus gp100 vaccine) would be the best.[37] However, study results showed no benefit associated with adding gp100.[37] In contrast, a trial of high-dose IL2 with or without gp100 found a higher ORR and longer PFS when gp100 was added to IL2, and a trend for OS benefit.[29]

Genome analyses have demonstrated that melanomas express hundreds to thousands of mutations,[51] many of which can produce mutated TAA.[52] Many of these mutated antigens, which are unique to each individual rather than shared, can be recognized by the immune system and effectively targeted with massive numbers of helper or cytotoxic T lymphocytes.[53,54] However, vaccination approaches with one or a few TAA or allogeneic cell lines have yielded disappointing clinical results,[55] and are unlikely to produce optimal immunization because of TAA heterogeneity among patients. For these reasons attention is focusing increasingly on autologous TAA.

Although inducing inflammation of an *in vivo* metastasis may enhance TAA recognition in some patients,[56,57] a better approach may be the use of pure autologous tumor cell lines as a source of TAA.[58] Use of autologous tumor cell lines may be the only way to capture unique TAA expressed on early self-renewing and proliferating tumor cells that make up a short-term cell line. This approach has all of the advantages of allogeneic cell lines combined with the autologous nature of the antigens, which overcomes the

Table 1. Common Features among Clinical Trials Testing Vaccines Derived from Autologous Tumor Cell Lines
Eligible patients had experienced distant metastatic melanoma or recurrent stage III melanoma.
A cell line had been established in the Hoag Cell Biology Laboratory from tissue obtained at the time of resection of a metastatic lesion.
Patients with hepatitis B or C, human immunodeficiency virus were not eligible.
Pregnant patients were not eligible.
Patients with known auto-immune disease were not eligible.
Patients had no significant hematologic, hepatic, or renal laboratory abnormalities.
Patients had good performance status (ECOG 0-1).
Patients originated from all over the U.S.
Patients with controlled brain metastases were eligible.
Patients were eligible regardless of whether they were anergic to standard skin tests.
Patients were referred for treatment by their managing physician.
At the time of treatment, patients were allowed to have no-evidence disease, detectable but non-measurable disease, or measurable disease.
Concurrent anti-cancer treatment was not allowed.
Patients were injected with a single subcutaneous injection of vaccine weekly for 3 weeks and then monthly for 4 months at weeks 8, 12, 16, 20, and 24.

limitations related to inter-patient heterogeneity and the negative effects of allogeneic antigens.[58,59]

Clinical Trials Using Vaccines Derived from Autologous Tumor Cell Lines

From 1990 to 2011 research teams working in the Hoag Cancer Center in Newport Beach, Calif., focused on growing autologous tumor cell lines for use as patient-specific vaccines.[60-67] Most of this work focused on patients with metastatic melanoma. Four sets of clinical data have been reported:

1. 74 patients injected with irradiated tumor cells (TC) with various adjuvants[65]
2. 54 patients injected with dendritic cells (DC) loaded with antigens from irradiated TC (DC-TC) and suspended in GM-CSF[66]
3. 42 patients treated in a randomized Phase II trial that compared DC-TC to TC, with both products suspended in GM-CSF[67]
4. A retrospective comparison of patients who were treated with IL2 or IL2 with an autologous TC or DC-TC vaccine before or after IL2.[34]

Critical eligibility criteria and features common to all 3 of these clinical trials are summarized in Table 1, above, and results of these trials are shown in Table 2, page 52. The most common toxicities were grade 1 or 2 local injection site reactions that occurred in about 75 percent of patients, similar to what is seen with single injections of GM-CSF. Objective tumor regressions were rare, as would be predicted for an immune effect targeting early proliferating cells more than differentiated tumor cells. Historical comparisons and the randomized trial suggested that the DC-TC product was associated with better OS than TC.[66,67] The effect on PFS was not nearly as impressive as the effect on OS. One durable complete response was noted, but could not be declared until nearly nine months after completion of therapy, after months of stable disease.[67,68] That patient previously had never been disease-free despite multiple surgeries, IL2, sorafenib and chemotherapy, and Gamma Knife treatment of brain metastases.

One question left unanswered was whether the apparent survival benefit associated with this therapy is dependent on tumor burden. In other words, is benefit seen both in patients who have no evidence of disease at the time of treatment and in those who have detectable disease at the time of treatment? To address this question, all 72 patients treated with DC-TC were compared to a more favorable subset of 71 of the 98 patients treated with TC. For patients who had no evidence of disease when treatment was started, 5-year survival rates were 73 percent for DC-TC (n=33) vs 43 percent for TC (n=37) (p=0.015).[69] The 43 percent survival rate for the TC arm is similar to that observed in other vaccine trials for patients who had been rendered disease free by surgery; 5-year OS rates were 40 to 45 percent for such patients treated with various peptide vaccines,[70] and BCG or BCG plus allogeneic tumor cells.[15] Among patients who had detectable disease, OS was again superior in the DC-TC arm (n=39) compared to TC (n=34),

Table 2. Results from Clinical Trials Testing Vaccines Derived from Autologous Tumor Cell Lines

NAME	74 TC	54 DC-TC	42 (DC-TC vs YC)
TRIAL	Phase I/II	Phase I/II	Phase II randomized
WHEN	1990–2001	2000–2006	2007–2011
ELIGIBILITY	• Metastatic melanoma • Successful TC line • MD decision to Rx	• Metastatic melanoma • Successful TC line • MD referral for Rx	• Metastatic melanoma • Successful TC line • MD referral for Rx
PRODUCT	Irradiated tumor cells (TC) as source of tumor-associated antigens (TAA)	DC loaded with TAA from irradiated autologous TC to produce DC-TC & suspended in GM-CSF	DC loaded with TAA from irradiated autologous TC to produce DC-TC & suspended in GM-CSF
PROTOCOL DESIGN AND # OF PATIENTS	Open label: up to 40 measurable patients and 40 non-measurable patients	Open label: up to 40 measurable patients and 40 non-measurable patients	Randomized, open label: 200 patients stratified by measurable disease and most advanced stage
PRIMARY EFFICACY ENDPOINTS	• Tumor skin test conversion • Objective response • Overall survival	• Tumor skin test conversion • Objective response • Overall survival	Overall survival α =p<.05, β =0 .80 40% difference, 2-tailed
ACCRUAL	• CBRG 90-08:TC-BCG (n=7) • CBRG 92-12 randomized phase II: TC + injections of GM-CSF v IFN-γ (n=38) • Compassionate use: other adjuvants (n=29)	• 15 measurable • 39 non-measurable	• Terminated early • 24 TC • 18 DC-TC
SCHEDULE	Subcutaneous weekly x 3 & monthly x 5	Subcutaneous weekly x 3 & monthly x 5	Subcutaneous weekly x 3 & monthly x 5
CELLS PER INJECTION	10 million (2 million to 24 million)	15 million (4 million to 35 million)	3 million DC-TC (5-23) 12 million TC (7-22)
MEDIAN AGE	50	51	DC-TC 58, TC 58
MALE : FEMALE	44:30	34:20	DC-TC 11:7, TC 16:8
HIGHEST STAGE EVER	Stage IV=44 (59%) Stage III=23 (31%) Unknown=7 (9%)	Stage IV= 44 (81%) Stage III= 10 (19%)	Stage IV=33 (79%) Stage III= 9 (21%)
STAGE @ Rx	Not adjusted for LDH	IIIa & Ib to Ic by LDH	IIIa & IVb to IVc by LDH
NED	35 (47%)	25 (46%)	19 (45%)
M1a	8 (11%)	3 (6%)	4 (10%)
M1b	13 (18%)	7 (13%)	6 (14%)
M1c	17 (22%)	19 (35%)	13 (31%)
% Rx AT HOAG	35/74 (47%)	54/54 (100%)	42/42 (100%)

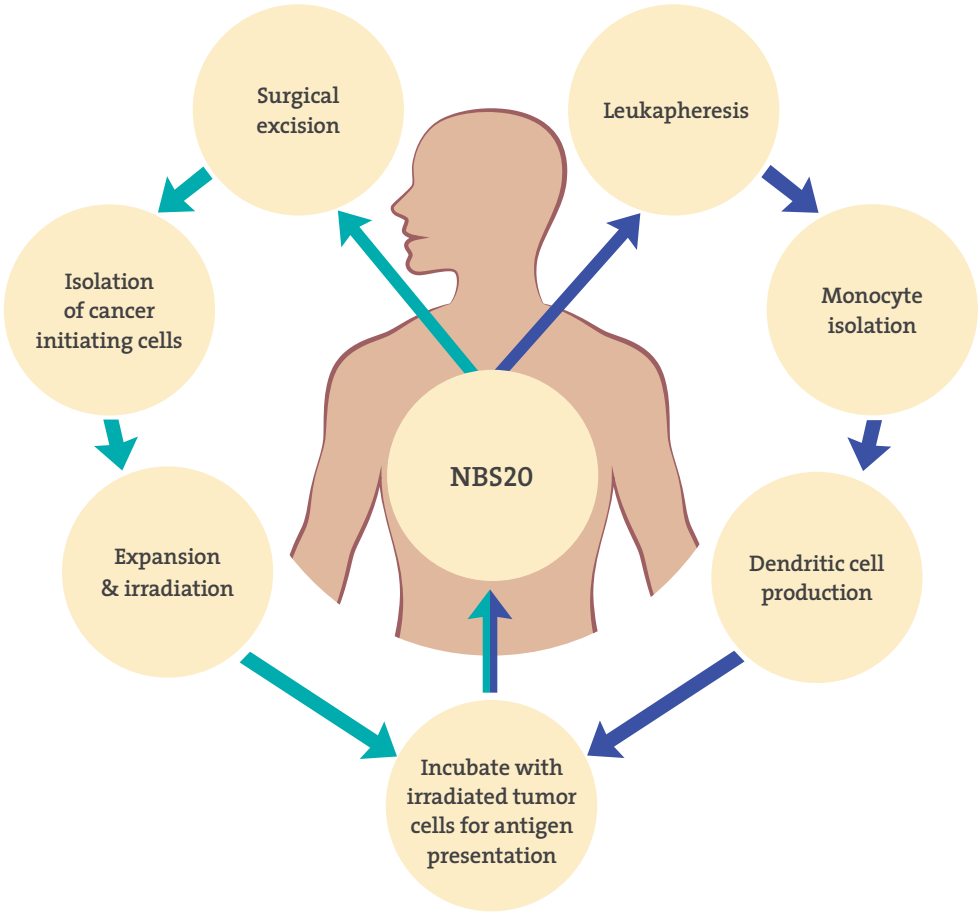
with a median OS of 39 vs 15 months, and 5-year OS of 33 percent vs 20 percent.[71] In a smaller subset of 32 patients who had measurable disease by RECIST criteria at the time of vaccine therapy, there was also a superior OS associated with DC-TC.[71]

Manufacturing NBS20, a DC-TC Candidate for Metastatic Melanoma

It is one scenario to develop a treatment such as this in a specialized translational research laboratory, but quite another scenario to make it a potential commercial product for practical delivery in the community. In other words, while research on NBS20

Figure 1. Sequence of Events Associated with the Creation of Patient-Specific NBS20

This schema illustrates the steps from tumor acquisition to treatment with patient-specific vaccine consisting of autologous dendritic cells loaded with antigens from an autologous tumor cell line, and injected s.c. (subcutaneous) with granulocyte-macrophage colony stimulating factor. The tumor cell production process takes about six weeks. The production of dendritic cells and loading with antigen takes about one week, and quality assurance procedures for product release take another two weeks.



began at the Hoag Cell Biology Laboratory, bringing it to market was another story. In 2011 California Stem Cell, Inc., Irvine, Calif., acquired Hoag Cell Biology Laboratory and the rights to NBS20. Then, in 2014, California Stem Cell was bought by NeoStem, Inc., N.Y. The sequence of events associated with the creation of each patient-specific product are summarized in Figure 1, above. The seven critical steps are:

1. ***Obtaining and shipping tumor tissue.*** Metastatic melanoma lesions are frequently resected as part of the standard of care, but for a biological product such as NBS20, the tissue must be collected in a manner that maintains sterility and viability, and processed in a manner that allows cryopreservation of cells that will be viable when thawed in the future, and/or processed for an effort to establish a tumor cell line.[72] To accomplish this, transport kits containing tissue culture media and antibiotics are provided. A viable portion of tumor tissue is selected by the surgeon and/or pathologist and sterilely placed into a media-containing vial, placed in the transport kit, and then sent by special delivery so that the tissue can be processed within 24 to 72 hours of the surgical resection. The quantity of tissue requested is about 1 cubic cm, but quality is more important than quantity. Viable well-vascularized tissue on the periphery of a mass is preferred to necrotic tissue; non-pigmented is preferred to pigmented tissue because melanin production is associated with more differentiated melanoma cells. A smaller lesion is preferred to a large lesion, because there may be a higher proportion of tumor stem cells or progenitor cells in a smaller lesion. Using these procedures, researchers have successfully established cell lines from tissues received up to 72 hours after resection and transported from Brazil, Switzerland, and Australia.

2. ***Processing tumor tissue.*** Once received, the tumor tissue has to be maintained under sterile conditions. Standard operating procedures are in place for digesting and mincing the tumor into cell suspension and placing cells into tissue culture for efforts to grow a cell line, or with DMSO (dimethylsulfoxide) and media for cryopreservation in the vapor phase of liquid nitrogen at less than -135° C.
3. ***Growing cell lines.*** The methods used in the Hoag Cell Biology Laboratory were not sufficient for a commercial product. Success rates over the years were about 50 percent for more than 600 specimens and were similar regardless of the cell biologists and laboratory technicians who worked with the samples.[65-67] It also took a long time to establish a cell line; the median time for success was about 4 months, with a range from 2 to 11 months.[73] As stated previously, in 2011 the assets and intellectual property of the Hoag Cell Biology Laboratory were acquired by California Stem Cell, Inc. The company applied its expertise in growing stem cells to increase the success rate and decrease the time required to establish tumor cell lines. In fact, cell lines have been established within 6 weeks from 80 percent of cryopreserved melanoma samples (personal communication with Andrew Cornforth of Stem Cell, Inc.), even though historically it took longer to grow a cell line from a frozen than fresh sample. This percentage has included successful growth of cell lines from samples that previously could not grow cell lines.
4. ***Irradiating tumor cells.*** Tumor cells are treated with high doses of radiation to inhibit the proliferative capability of the cells to reduce the slim chance that viable tumor cells might be injected back into the patient. Such radiation also induces apoptosis in a manner that facilitates phagocytosis and antigen processing by DC. Proteins are partially digested and then expressed on the surface of the DC in the context of histocompatibility molecules to initiate a new anti-TAA immune response or enhance an existing immune response.
5. ***Collecting peripheral blood mononuclear cells (PBMC).*** Dendritic cells (DC) are derived from PBMC. DC are now appreciated as being the most efficient of the antigen presenting cells (APC) that communicate with T cells in the adaptive immune system. Animal and human studies suggest that TAA presentation by DC that have been loaded with antigen *ex vivo*, result in better immune responses and better clinical outcome than simply injecting TAA with a cytokine or adjuvant.[67] PBMC are collected in the process of leukapheresis (a procedure in which white blood cells are separated from a sample of blood) that is performed using machines designed for collection of different blood elements and plasma on the basis of differential centrifugation. Many physicians are familiar with leukapheresis because of collection of hematopoietic stem cells for autologous or allogeneic bone marrow transplants, the intravenous dendritic-cell immunotherapy sipuleucel-T for prostate cancer,[74] and various vaccine clinical trials that require generation of DC. The procedure itself typically involves a 10 liter exchange over four to five hours. Good venous access is required so that blood can be removed from the body, PBMC segregated and removed, and the rest of the blood product returned to the patient. Patients must have adequate veins to withstand the draw pressures so the veins do not collapse. When collecting PBMC for autologous or allogeneic transplants, central lines are required in most patients because of the draw pressures. Fortunately, central lines are generally not required when collecting PBMC from which to generate DC.

   During leukapheresis, anti-coagulation with citrate is required to avoid clotting, and it can cause symptomatic hypocalcemia, especially in patients with mild Vitamin D deficiency, such as that commonly associated with metabolic syndrome. Mild symptoms such as perioral (around the mouth) tingling are usually easily controlled with calcium carbonate (e.g., Tums®) and/or milk products. Intravenous calcium chloride may be required for patients that have more severe or persistent symptoms of hypocalcemia.

   For multicenter trials, PBMC can be collected by any appropriately certified pheresis facility, placed in a transfer kit, and then shipped to the NeoStem facility in Irvine, Calif. (formerly California Stem Cell, Inc.). Many cancer programs have their own leukapheresis facilities, especially if they are involved in bone marrow transplants or cell-based biological therapies. However, there are commercial pheresis entities that provide this service, including the American Red Cross, HemaCare, and Blood Centers of America. In contrast to the sipuleucel-T product for prostate cancer that requires three leukaphereses,[74] only one pheresis procedure is needed to derive enough cells for all eight planned injections of NBS20. Further purification of the PBMC and growth in interleukin-4 and GM-CSF results in production of immature DC in about 6 days.
6. ***Combining DC and TC.*** NBS20 (DC-TC) consists of autologous DC cells loaded with TAA from the irradiated autologous TC by co-incubation for 12 to 18 hours. During this time DC phagocytose (engulf and destroy) the TC and present antigenic fragments in the context of HLA histocompatibility proteins for presentation to T lymphocytes. Each dose contains TAA derived from about 10 million self-renewing, proliferating, autologous TC. The loading process is associated with maturation of DC, which helps optimize presentation of TAA to T lymphocytes. Quality testing for product release currently requires an additional two weeks. The final product is divided into aliquots containing 5 million to 20 million cells for each of the intended 8 injections and stored in a cryovial. The time from leukapheresis to availability of NBS20 for treatment is 4 weeks, or about 1 month.
7. ***Storage, preparation, and administration of NBS20.*** All doses are shipped in a cryopreserved state to the treatment site for storage in the vapor phase of liquid nitrogen in a dewar (a tank designed for this purpose) which needs to be at or very near the treatment site. There are companies that provide a refill service to maintain the desired liquid nitrogen level for the dewar. Alternatively, it is possible to send each dose in its own dewar containing sufficient liquid nitrogen to last for several days. The cell product is maintained in this manner until just prior to administration, when one conical vial is thawed at room temperature (approximately 68°-75° F, 20°-24° C) under

a sterile hood. Next, 500 microgram of GM-CSF is reconstituted in 0.5 ml of saline and injected into the cryovial to suspend the DC-TC product. The final 1.1 ml volume of GM-CSF and DC-TC is drawn into a 3.0 ml syringe and 1.0 ml of liquid and cells is injected subcutaneously via 25-gauge needle into one of the patient's extremities for each administration. Once thawed, the cell product should be injected as soon as possible, and within five hours.

The INTUS Trial

The U.S. Food and Drug Administration (FDA) granted NBS20 orphan drug status and a special protocol assessment and fast track designation in 2013. (Breakthrough status was not warranted because there is no standard therapy for comparison that is recognized as adjunctive treatment for patients with metastatic melanoma.) GM-CSF has been used in similar patients, but clinical benefit from this approach was not confirmed in randomized trials.[75, 76]

The INTUS trial, NCT01875653, which opened for enrollment in late October 2014, is a double-blinded, placebo-controlled, randomized trial for patients with distant metastatic melanoma or recurrent stage III melanoma. The randomization is 2:1 for the study agent NBS20 to control. The plan is to randomize and treat 250 patients. The control arm is autologous monocytes (MC) in order to facilitate the double-blind design. Leukapheresis is performed shortly after randomization to collect PBMC from which DC or MC are derived. Both treatment products, DC-TC and MC, become available about one month after leukapheresis, and are suspended in 500 µg GM-CSF for injection. Entry criteria are similar to those used in the previous trials as summarized in Table 1, page 51. There are no restrictions related to prior or subsequent therapies, but concurrent therapy is not allowed. Managing physicians and patients should recognize that pre-enrollment screening can take up to a month, and it takes another month from the time of randomization and leukapheresis to availability of the treatment product.

Patients are stratified based on the extent of disease at the time of randomization as follows:

1. No evidence of disease
2. Presence of non-measurable or equivocal disease
3. Measurable disease with a serum lactate dehydrogenase (LDH) that is in the normal range
4. Measurable disease with an elevated LDH.

RECIST criteria are used to define the appropriate strata for each patient,[77] but determination of ORR or PFS are not endpoints for this trial. Based on theoretical considerations, and observations made in earlier trials, the only endpoint is death for determination of OS. If most of the anti-tumor effect is on the small number of tumor stem cells present in various lesions, then a response can only be determined once more differentiated cells that do not express these antigens have ceased replicating and die off; therefore, objective responses are likely to be rare, and delayed, which is consistent with what has been seen in previous trials.

Similarly, if we are targeting a small population of cells in a given tumor mass, untargeted cells will continue to grow and the lesion is likely to enlarge for a period of time until the more differentiated tumor cells die off; therefore, PFS is unlikely to be prolonged, which is consistent with what was observed in earlier trials. Targeting a small subset of such cells can eliminate established tumors in animal models.[78] Even though OS potentially could be confounded by other therapies, it is the only meaningful endpoint for an immune response that should persist for many years, if not indefinitely; therefore a randomized, double-blinded, placebo-controlled trial with overall survival as the endpoint is the appropriate study design. OI

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AS OUR UNDERSTANDING OF TUMOR BIOLOGY and microenvironment evolves and an increasing number of immunotherapy approaches become available in oncology, immuno-oncology therapy is poised to revolutionize patient care by developing therapies that put the body's own immune system to work to fight cancer. In 2014 the Association of Community Cancer Centers established the Institute for Clinical Immuno-Oncology (ICLIO) to educate providers about immuno-oncology and its implementation and delivery in the community setting. The project is made possible by a charitable donation from Bristol-Myers Squibb.

To learn more about ICLIO's clinical scholars engagement program, monthly e-newsletter, series of educational webinars, and national education conference, Oct. 2, 2015, Philadelphia, Pa., go to www.accc-cancer.org/ICLIO or email Lorna Lucas, MS, ACCC's senior manager for ICLIO at llucas@accc-cancer.org.