Adoptive Cell Transfer and Lymphodepleting Chemotherapy for the Treatment of Metastatic Melanoma

Tad W. Westdale
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Adoptive Cell Transfer and Lymphodepleting Chemotherapy for the Treatment of Metastatic Melanoma

Abstract
Introduction: Malignant melanoma, the most aggressive of all skin cancers, is on the increase throughout the world. Melanoma is a malignant tumor of melanocytes predominately of skin, but also found in the digestive tract and eye. Its occurrence is statistically less than other forms of skin cancer, but corresponds to the highest mortality. Surgical resection, in its early stages, is the only proven curative treatment. Metastatic disease is incurable in most patients due to its refractibility to systemic cancer treatment. There are currently a large number of treatment regimens in clinical use, and also ongoing clinical trials. The problem with currently available regimens relates to the high cost and relative toxicities. However, in the last decade, trials utilizing adoptive transfer of lymphocytes after host lymphodepletion via chemotherapy, have demonstrated objective cancer regression in human patients with metastatic melanoma.

Methods: The focus of this study was to review the current literature for the last 10 years on all studies pertaining to Adoptive Cell Transfer in treating metastatic melanoma. Electronic databases (Ovid – Medline, Pub-Med, MD Consult, NIH) were utilized. There were no gender or age restrictions. Only studies published in English were reviewed.

Results: Four clinical studies were chosen for this review in which patients with refractory metastatic malignant melanoma were treated with varying doses of lymphdepleting chemotherapy and ACT. Variables within each study were manipulated as to ascertain response changes, but the primary treatment remained the same. Favorable clinical response was observed in a minority of patients in each study, but because of the experimental nature of this method of treatment, overall outcomes continue to fluctuate.

Conclusion: This new form of epithelial cancer treatment is just in its infancy. With each consecutive research trial, advances continue to be made. Melanoma is the deadliest of all skin cancers. With advances being made daily in cellular engineering, it is only a matter of time before we have an effective form of ACT treatment for malignant melanoma.

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Adoptive Cell Transfer and Lymphodepleting Chemotherapy for the Treatment of Metastatic Melanoma

Tad W. Westdale

A Clinical Graduate Project Submitted to the Faculty of the
School of Physician Assistant Studies
Pacific University
Hillsboro, OR
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Faculty Advisor: Judy Ortiz MS, MHS, PA-C, Assistant Director SPAS
Clinical Graduate Project Coordinators: Rob Rosenow PharmD, OD & Annjanette Sommers MS, PA-C
Biography

Tad Westdale was born in Grand Rapids, Michigan. As a boy, he was fortunate to live in a rural area where he learned to appreciate nature. Fish, turtles, frogs, snakes, and his trusty four legged companion, Brandy, were the focus of his days. His family then moved to the suburbs where he found a love for sports and good friends. Unfortunately, he lost his father at the age of forty three to cancer. From that time on, he has focused on medicine as his life’s profession.

At the age of eighteen, he went west to attend the University of Montana, where he attained a Bachelor of Science in Exercise Science. During his undergraduate studies, he began to work for the county ambulance as an EMT. This was an exciting time that offered many opportunities. He eventually became a Paramedic and met his wife Stacey. She also was a Paramedic who shared many common interests with him. Skiing down the mountains, fly fishing a quiet stream, and enjoying a well deserved view after a long hike were some of the many things they enjoyed while living in Montana.

Looking for greener pastures, and graduate schooling, the two moved to southwest Washington. Both were able to obtain jobs as Paramedics, but refused to work together (for better or worse, but not for lunch). Following many years of Emergency Medical Service, Tad began his studies to become a Physician Assistant at Pacific University in Hillsboro, Oregon.

He currently is putting the finishing touches on completion of a Masters degree in Physician Assistant Studies, and will soon be prospecting for a job. But at the end of the day he most enjoys relaxing with his wife and two dogs.
Abstract

**Introduction:** Malignant melanoma, the most aggressive of all skin cancers, is on the increase throughout the world. Melanoma is a malignant tumor of melanocytes predominately of skin, but also found in the digestive tract and eye. Its occurrence is statistically less than other forms of skin cancer, but corresponds to the highest mortality. Surgical resection, in its early stages, is the only proven curative treatment. Metastatic disease is incurable in most patients due to its refractibility to systemic cancer treatment. There are currently a large number of treatment regimens in clinical use, and also ongoing clinical trials. The problem with currently available regimens relates to the high cost and relative toxicities. However, in the last decade, trials utilizing adoptive transfer of lymphocytes after host lymphodepletion via chemotherapy, have demonstrated objective cancer regression in human patients with metastatic melanoma.

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**KEYWORDS:** Adoptive Cell Transfer, Chemotherapy, Melanoma, Treatment, Immunotherapy
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<td>TIL</td>
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Adoptive Cell Transfer and Lymphdepleting Chemotherapy for the Treatment of Metastatic Melanoma

Introduction

Malignant melanoma (MM) develops due to changes in the melanocyte cells that produce melanin pigment. Melanocytes are most commonly found within the skin, but are also present in the uveal tract, upper digestive tract, anal canal, rectum, and vagina. One in six Americans will develop skin cancer at some point in their life.¹ Skin cancer accounts for one third of all cancers in the United States.¹ Around 160,000 new cases of melanoma are diagnosed worldwide each year.² MM is more common in Caucasian populations living in sunny climates than in other groups.³ According to a World Health Organization report, about 48,000 melanoma related deaths occur worldwide per year.⁴ Fortunately, most patients with skin cancer develop non-melanoma lesion(s), and mortality secondary to these lesions is rare. Metastatic Malignant Melanoma (MMM), however, accounts for 75% of all deaths associated with skin cancer.¹

In contrast to non-melanoma skin cancer (basal cell and squamous cell carcinoma), which typically affects older individuals, the frequency of MM peaks between 20 and 45 years of age.⁵ Mortality rates are higher in men than in women.⁵ This occurrence is likely due to unrecognized lesions which develop in less easily observed areas, such as the back in men, and the dorsal leg in women (Figure I). Mortality is also increased in blacks, as is the propensity to develop more aggressive tumors and to be diagnosed at later stages.⁶

Almost all malignant melanomas start with altering the color and appearance of normal-looking skin. The area may be an abnormally darkly pigmented nevus, a new nevus, or one that has recently changed. It can be very difficult to distinguish the difference between a malignant
melanoma and a normal nevus. When looking for danger signs in pigmented lesions of the skin, the “ABCDE” mnemonic is utilized (Table I): **Asymmetry** – Melanomas most likely have an irregular or asymmetrical shape. Typical nevi are usually symmetrical. **Border** – Melanomas may have poorly defined borders or ridged edges. Normal nevi have defined smooth edges. **Color** – Melanomas have many colors. They may contain a few different shades such as brown mixed with black, red, pink, white or a bluish tint. Normal nevi are regularly one shade of brown. **Diameter** – Most melanomas are more than 7mm in diameter, but many patients have been diagnosed with lesions much smaller. **Evolving (changing)** – Nevi may change in their size, shape or color which is a vital sign of malignant melanoma.\(^7\)

Features that affect prognosis are, tumor thickness in millimeters (Breslow's depth: Table III), depth related to skin structures (Clark level: Table III), type of melanoma, presence of ulceration, presence of lymphatic/perineural invasion, location of lesion, presence of satellite lesions, and presence of regional or distant metastasis.\(^8\)

Certain types of melanoma have worse prognoses but this is explained by their thickness. Interestingly, superficial melanomas even with lymph node metastases carry a better prognosis than deep melanomas without regional metastasis at the time of staging. The presence of tumor infiltrating lymphocytes at the site of the lesion has also been correlated to an improved prognosis. Local recurrences tend to behave similarly to a primary lesion unless they are at the site of a wide local excision (as opposed to a staged excision or punch/shave biopsy) since these recurrences tend to indicate lymphatic invasion.\(^8\)

When a melanoma spreads to a lymph node, one of the most important factors is the number of nodes affected. Extent of malignancy within a node is also important; micrometastases in which malignancy is only microscopic have a more favorable prognosis than
macrometastases. In some cases micrometastases may only be detected by special staining, and if malignancy is only detectable by a rarely-employed test known as the polymerase chain reaction (PCR), the prognosis is even better. Macrometastases in which malignancy is clinically apparent (in some cases the cancer may completely encompass a node) have a far worse prognosis, and if nodes are matted or if there is extracapsular extension, the prognosis is dire.9

When there is distant metastasis, the cancer is generally considered incurable. The five year survival rate is less than 10%. The median survival is 6 to 12 months. Treatment is palliative, focusing on life-extension and quality of life. In some cases, patients may live many months or even years with metastatic melanoma. Metastases to skin and lungs have a better prognosis, while metastases to brain, bone, and liver are more difficult to treat.9

There is not enough definitive evidence to adequately stage, and thus give a prognosis for ocular melanoma and melanoma of soft parts, or of mucosal melanomas (like rectal melanoma), although these tend to metastasize more aggressively. Even though regression may increase survival, when a melanoma has regressed, it is impossible to know its original size and thus the original tumor is often more widespread than a pathology report might indicate.9

Sometimes the skin lesion may bleed, itch, or ulcerate, although this is a very late sign. A slow-healing lesion should be watched closely, as that may be a sign of melanoma. Be aware also that in circumstances that are still poorly understood, melanomas may "regress" or spontaneously become smaller or invisible - however the malignancy is still present.9

**Genetics**

Familial melanoma is genetically heterogeneous,10 and loci for familial melanoma have been identified on the chromosome arms 1p, 9p and 12q. Multiple genetic events have been related to the pathogenesis of melanoma.11 The multiple tumor suppressor 1 (CDKN2A/MTS1)
gene encodes p16INK4a - a low-molecular weight protein inhibitor of cyclin-dependent protein kinases (CDKs) - which has been localized to the p21 region of human chromosome 9.\textsuperscript{12} Currently, melanomas are diagnosed only after they become visible on the skin. In the future, however, physicians will hopefully be able detect melanomas based on a patient’s genotype, not just his or her phenotype. Recent genetic advances promise to help identify people with high-risk genotypes and to determine which of a person’s lesions have the greatest chance of becoming cancerous. A number of rare mutations, which often run in families, are known to greatly increase one’s susceptibility to melanoma. People with mutations in the MC1R gene, for example, are two to four times more likely to develop melanoma than those with two wild-type copies of the gene. MC1R mutations are very common; in fact, all people with red hair have a mutated copy of the gene. In addition to identifying high-risk patients, researchers also want to identify high-risk lesions within a given patient. Many new technologies, such as optical coherence tomography (OCT), are being developed to accomplish this. OCT allows pathologists to view 3-D reconstructions of the skin and offers more resolution than past techniques could provide.\textsuperscript{12}

**Prevention**

Minimizing exposure to sources of ultraviolet radiation is necessary for prevention of malignant melanoma. There is a health campaign slogan in Australia which states “Slip-Slop-Slap”. The goal of this country’s most recognized health message is to have people *slip* on a shirt, *slop* on sunscreen, and *slap* on a hat.

In the past, it was recommended that people use sunscreens with an SPF rating of 30 or higher on exposed areas as older sunscreens more effectively blocked UVA with higher SPF.
Currently, newer sunscreen ingredients (avobenzone, zinc, and titanium) effectively block both UVA and UVB even at lower SPFs. However, there are questions about the ability of sunscreen to prevent melanoma. This controversy is discussed in numerous review articles, but dermatologists continue to take the position that the use of sun screen of at least 30 SPF is necessary.\textsuperscript{13} This correlation might be due to the confounding variable that individuals who used sunscreen to prevent burn might have a higher lifetime exposure to either UVA or UVB.

Tanning, once believed to help prevent skin cancers, actually can lead to increase incidence of melanomas.\textsuperscript{13} Even though tanning beds emit mostly UVA, which causes tanning, it by itself might be enough to induce melanomas. This risk factor should be stressed to patients who frequently \textit{bask under the bulbs}.

A good rule of thumb for decreasing ultraviolet light exposure is to avoid the sun between the hours of 9 a.m. and 3 p.m during the summer solstice, or to avoid the sun when your shadow is shorter than your height. These are rough rules, however, and can vary depending on locality and individual skin cancer risk.\textsuperscript{13}

\section*{Treatment}

Surgery is the first choice therapy for localized cutaneous melanoma. Depending on the stage, a sentinel lymph node biopsy is done as well. Treatment of advanced malignant melanoma is performed with a multidisciplinary approach.

Complete surgical excision with adequate margins and assessment for the presence of detectable metastatic disease along with aggressive follow up is standard.\textsuperscript{14} The surgery involves making a wide local excision (WLE) with 1 to 2 cm margins. Melanoma-in-situ is treated with
narrower surgical margins, usually 0.2 to 0.5 cm. Many surgeons consider 0.5 cm the standard of care for excision of melanoma-in-situ, but 0.2 cm margins might be acceptable for margin controlled surgery such as Mohs’ micrographic surgery.14 The WLE aims to reduce the rate of tumor recurrence at the site of the original lesion. This is a common pattern of treatment failure in melanoma. Following definitive diagnosis of melanoma, Mohs’ surgery has been reported with cure rate as low as 77% and as high as 98% for melanoma-in-situ.14,15 Failure to remove sufficient tissue due to incorrect margins is a common reason for treatment failure in malignant melanoma.

Melanomas which spread usually do so to the lymph nodes in the region of the tumor before spreading elsewhere. Attempts to improve survival by removing lymph nodes surgically (lymphadenectomy) were associated with many complications but unfortunately with no overall survival benefit.16 Recently the technique of sentinel lymph node biopsy has been developed to reduce the complications of lymph node surgery while allowing assessment of the involvement of nodes with tumor.16

Although controversial and without prolonging survival, sentinel lymph node (SLN) biopsy is often performed, especially for T1b/T2+ tumors, mucosal tumors, ocular melanoma, and tumors of the limbs.17 A process called lymphoscintigraphy is performed in which a radioactive tracer is injected at the tumor site in order to localize the sentinel nodes. Further precision is provided using a blue tracer dye and surgery is performed to biopsy the nodes. Routine haematoxylin and eosin staining, and immunoperoxidase staining are adequate to rule out node involvement.17 Polymerase chain reaction (PCR) tests on nodes, usually performed to test for entry into clinical trials, now demonstrate that many patients with a negative SLN
actually had a small number of positive cells in their nodes. Alternatively, a fine-needle aspiration may be performed and is often used to test masses.\textsuperscript{18} When a lymph node displays malignancy, a radical lymph node dissection will often be performed. If the disease is completely resected, the patient will be considered for adjuvant therapy.\textsuperscript{17}

**Adjuvant treatment**

High risk melanomas typically require adjuvant treatment. In the United States most patients in otherwise good health, will begin up to a year of high-dose interferon treatment, which has severe side effects but may improve the patients’ prognosis.\textsuperscript{19} This claim is not supported by all research at this time, and in Europe interferon is usually not used outside the scope of clinical trials.\textsuperscript{20,21}

Metastatic melanomas can be detected by X-rays, CT scans, MRIs, PET and PET/CTs, ultrasound, LDH testing, and photo-acoustic detection.\textsuperscript{22} Lactate dehydrogenase (LDH) tests are also often used to screen for metastases, although many patients with metastases, even end-stage, have a normal LDH. Extraordinarily high LDH often indicates metastatic spread of the disease to the liver.\textsuperscript{22}

Various chemotherapy agents are used, including dacarbazine (also termed DTIC), immunotherapy (with interleukin-2 (IL-2) or interferon (IFN)) as well as local perfusion are used by different centers. They can occasionally show dramatic success, but the overall success in metastatic melanoma is quite limited.\textsuperscript{23} IL-2 (Proleukin) is the first new therapy approved for the treatment of metastatic melanoma in 20 years. Studies have demonstrated that IL-2 offers the possibility of a complete and long-lasting remission in this disease, although only in a small
percentage of patients. A number of new agents and novel approaches are under evaluation and show promise.

On June 23, 2008, Israeli scientists from the Oncology Institute of the Hadassa Medical Center in Jerusalem announced, that they had developed a vaccine by utilizing tumor surface antigens to stimulate the patient’s own immune response. This therapy apparently prevented recurrences of the disease among those in remission, and displayed increased survival for patients with active disease.

**Recent Approaches in Treatment**

Experimental treatment developed at the National Cancer Institute (NCI), part of the National Institutes of Health in the US, was used in advanced (metastatic stage III/IV) melanoma, with moderate success. The treatment is adoptive transfer of genetically altered autologous lymphocytes which are reintroduced into the patient following a course of lymphodepleting chemotherapy. After manipulation and re-population, the lymphocytes recognize and bind to tumor-associated antigens (TAA) found on the surface of melanoma cells and destroy them. The genes encoding T-cell receptors (TCR) that are specific for a variety of TAA have now been cloned and are being utilized in treatment. These TCRs have been shown to react to approximately 50% of cancers arising from common epithelial origins (which Dudley et al. and colleagues observed).

Adoptive cell therapy (ACT) has emerged as the most promising treatments for patients with metastatic melanoma. ACT-based immunotherapy was first described in 1988, but the decisive improvement in efficacy came in 2002 with the introduction of an immunodepleting
preparative regimen given before the adoptive transfer. This treatment adjunct improved the repopulation of the patient’s anti-tumor T cells.\textsuperscript{28} Of patients with MMM refractory to all other treatments, 50% experienced an objective response, some with complete responses.\textsuperscript{29} Recent studies demonstrating that normal human lymphocytes can be genetically engineered to recognize cancer antigens and to mediate cancer regression \textit{in vivo}, has opened doors for enhancing and extending the ACT approach to patients with a wide variety of cancer types.\textsuperscript{30}

This breakthrough researcher brings up the clinical question: Is there a benefit to treatment with ACT following lymphodelpletting chemotherapy for the treatment of patients with refractory metastatic melanoma?

\textbf{Materials and Methods}

A comprehensive literature search was employed by utilizing the following internet databases and websites: Ovid:Medline, Pub Med, The Journal of Clinical Oncology, and the National Institutes of Health. The keywords: Adoptive cell transfer, melanoma, treatment, and chemotherapy were entered for search purposes.

Inclusion criteria considered were literature, in the English language, from January 1999 to present was focused upon for the determination of ACT therapy. Those articles chosen were compiled and analyzed to determine relevance to addressing the clinical question.

Studies which incorporated animal subjects, vaccine administration, myeloablative chemotherapy, and interferon administration were excluded. Clinical trials prior to January 1999 were also excluded. Studies not published in English were not utilized. Jadad score was applied to each article, but little benefit was observed due to the nature of the clinical trial design.
Results

Four articles, published 2002-2008, were the subject of this review to determine risk/benefit and overall survival of treatment with ACT/chemotherapy for patients with MMM. These articles afforded the opportunity to draw a conclusion as to the future of ACT therapy.

A phase I clinical trial using non-myeloablative, lymphodepleting chemotherapy in combination with adoptive immunotherapy in patients with MMM. The chemotherapy-conditioning schedule that induced transient lymphopenia consisted of cyclophosphamide (30 or 60 mg/kg per day for 2 days) followed by fludarabine (25 mg/m² per day for 5 days).

Immunotherapy for all patients consisted of in vitro expanded, tumor-reactive, autologous T-cell clones selected due to their ability to recognize melanoma surface antigens. Cohorts of three to six patients each received either no interleukin (IL)-2, low-dose IL-2 (72,000 IU/kg intravenously three times a day to a maximum of 15 doses), or high-dose IL-2 (720,000 IU/kg intravenously three times a day for a maximum of 12 doses). The toxicities associated with this treatment were transient and included neutropenia and thrombocytopenia that resolved in all patients. Interestingly, administration of high dose intravenous IL-2 was better tolerated by patients after chemotherapy than during previous immunotherapy cycles without chemotherapy.

The results revealed that not one of the fifteen patients exhibited an objective clinical response to treatment, although five patients demonstrated mixed responses or transient shrinkage of metastatic tumors. These findings led the researchers to believe that cellular persistence may be an extremely important component to effective treatment. Dudley et al. and colleagues established that a non-myeloablative conditioning regimen could be safely administered in conjunction with ACT and IL-2 in patients with metastatic melanoma.
The research trial which stimulated medical community awareness in ACT was published on April 1, 2005 in the Journal of Clinical Oncology. The primary research coordinator was Mark E. Dudley in conjunction with the National Institutes of Health (NIH). This team conducted a follow-up study to the one they had conducted in 2002. The trial investigated the combination of lymphodepleting chemotherapy followed by the adoptive transfer of autologous tumor reactive lymphocytes for the treatment of patients with refractory metastatic melanoma. The study trial consisted of thirty-five patients with MM, all but one with disease refractory to treatment with high-dose interleukin (IL-2), and many with progressive disease after chemotherapy. These subjects underwent lymphodepleting conditioning with two days of cyclophosphamide (60mg/kg) followed by five days of fludarabine (25 mg/m²). The day following the final dose of fludarabine, all patients received cell infusion with autologous tumor-reactive, rapidly expanded tumor infiltrating lymphocyte cultures and high-dose IL-2 therapy.

The results of this treatment regimen resulted in eighteen of thirty-five (51%) patients experiencing objective clinical responses which included three ongoing complete responses and fifteen partial responses with a mean duration of 11.5 +/- 2.2 months. Sites of regression included, metastases to lung, liver, lymph nodes, brain, and cutaneous and subcutaneous tissues. Toxicities of treatment included the hematologic toxicities of chemotherapy including neutropenia, thrombocytopenia, and lymphopenia (the transient toxicities of high-dose IL-2 therapy). Two patients developed Pneumocystis pneumonia, and one patient developed an Epstein-Barr virus–related lymphoproliferation. The research team concluded the lymphodepleting chemotherapy followed by the transfer of highly aggressive antitumor lymphocytes (ACT) can mediate significant tumor regression in heavily pretreated patients with...
IL-2 refractory MM. Although the study’s findings showed statistically significant results, the administration of IL-2 was also shown to produce systemic toxicities, which complicated the patient’s prognosis.

Another follow-up study published in May 2008, conducted by the NIH, focused upon the ACT of genetically engineered TILs which produced IL-2 with the promising conclusion that this method of therapy would be just as efficacious as the previously used high dose supplement of IL-2, without the toxic effects to the patients (Dudley et al. and colleagues 2008).

Thirteen patients received non-myeloablative, lymphodepleting chemotherapy, as previously described by Dudley et al. 2005, consisting of two days of cyclophosphamide (60 mg/kg) followed by five days of fludarabine (25 mg/m2). One day after the final dose, patients received IL-2-transduced TILs via intravenous infusion over thirty minutes. From this point in the trial, the patients were randomly split into three cohorts to receive slightly different therapy to determine the efficacy of supplemental IL-2. In cohort I, TILs from three patients were transduced twice (using $3 \times 10^6$ cells per well), followed by one rapid expansion with OKT3 antibody (30 ng/ml; Ortho Biotech, Bridgewater, NJ), a 200-fold excess of irradiated (4000 rad) allogeneic feeder PBMCs, and IL-2 (6000 IU/ml) in 50/50 medium [50% CM mixed with 50% AIM-V (Invitrogen)], which typically resulted in a 1000-fold expansion in fourteen days.

In cohort II, the five patient’s TILs were transduced during days seven and eight of the first rapid expansion (using $3 \times 10^6$ cells per well) followed by a second expansion to obtain sufficient cell numbers for treatment. Patients in cohorts I and II did not receive exogenous IL-2. The remaining five patients in cohort III were TIL transduced on days three and four of the first expansion (using $1 \times 10^6$ cells per well), followed by a second expansion, and the patients in this
cohort did receive concomitant high-dose IL-2 injections (720,000 IU/kg intravenously) every eight hours until tolerance developed.

The outcomes of this study were slightly discouraging with only two patients who exhibited partial response up to four to six months (one patient from cohort II and one patient from cohort III). Because TILs are mainly CD8+ T cells, which have a much lower capacity for IL-2 secretion, the need for CD4+ T cell help (Janssen et al., 2003; Antony et al., 2005; Williams et al., 2006) might be circumvented in part by endowing the CD8+ T cell with IL-2 production. The authors deduced that the low response rate was due to factors involved with the genetic engineering of the TILs.

A study conducted by the NIH, published in October of 2006, reached success in the use and application of immunotherapy through the ACT of lymphocytes after host immunodepletion. The researchers determined that, it could be possible to mediate objective cancer regression in human patients with MMM. This study was headed by Richard A. Morgan and Mark E. Dudley, whose team was able to report the ability to specifically confer tumor recognition by autologous lymphocytes from peripheral blood by using a retrovirus that encodes a TCR, specifically the MART-1 TCR. All 17 patients selected had progressive MMM, refractory to previous therapy with IL-2.

The patients in this study were divided into three cohorts and received ACT with varying \textit{ex vivo} culture periods. One goal of the study was to determine if the growth phase and culture periods had an effect on cell persistence \textit{in vivo}. The other, was to determine treatment response. The three patients in Cohort I were administered ACT following nineteen days of \textit{ex vivo} culture. These patients displayed poor cell persistence in vivo, and no tumor response was noted in any patient. Ten patients in Cohort II received ACT following six to eight day of \textit{ex vivo} culture. All
patients had evidence of cell persistence upwards of ninety days. Unfortunately, only one patient had a response to treatment, and it was only a partial response. The third cohort consisted of four patients who were administered ACT following eighteen days of *ex vivo* culture with the addition of a rapid expansion protocol. This group also showed MART-1 CTLs at greater than ninety days post administration, but again only one patient displayed any clinical response to treatment. All clinical responses were judged by Response Evaluation Criteria in Solid Tumors (RECIST).

Adoptive transfer of the transduced cells in 14 patients resulted in durable engraftment at levels exceeding 10% of peripheral blood lymphocytes for at least two months after the infusion. They observed high sustained levels of circulating, engineered cells at one year after infusion in 2 patients who both demonstrated objective regression of MMM lesions. No toxicities in any patient could be attributed to the modified CTLs or ACT protocol.

Patient 4 (cohort II), a 52-year-old male, had previously received treatment with interferon-α (IFN-α), a lymph node dissection, an experimental vaccine, and high-dose IL-2. The patient then developed progressive disease in the liver and axilla. After treatment with the ACT protocol, he experienced complete regression of the axillary mass and an 89% reduction of the liver mass, at which time it was removed. He remained clinically disease-free at twenty one months after treatment. Patient 14 (cohort III), a 30-year-old male, previously had received treatment consisting of a lymph node dissection, IFN-α, and high-dose IL-2. He developed an enlarging mass in the lung hilum. After ACT treatment, his hilar mass regressed and he remained clinically disease-free at 20 months post. Thus, two patients with rapidly progressive metastatic melanoma showed full clinical regression of disease after the transfer of genetically engineered autologous PBLs, which delighted Dudley et al. and colleagues.
A clinical trial published in October of 2000 by Cassian Yee et al. and colleagues inspected a current strategy for immunotherapy in treating MMM. The focused treatment of this study incorporated the augmentation of the immune response to tumor antigens represented by TCR proteins such as tyrosinase, gp100, and MART-1. The possibility that intentional targeting of tumor antigens representing normal proteins can result in autoimmune toxicity has been postulated but never demonstrated.

In this study, a single patient with MMM developed inflammatory lesions (circumscribed, pigmented areas of skin) after an infusion of MART-1–specific CD8+ T cell clones. Analysis of the TILs in skin and tumor biopsies using T cell–specific peptide–major histocompatibility complex (MHC) tetramers demonstrated a localized predominance of MART-1–specific CD8+ T cells (>28% of all CD8 T cells) that was identical to the infused clones. In contrast to skin biopsies obtained from the patient before T cell infusion, post-infusion tissue biopsies demonstrated the loss of MART-1 expression, evidence of melanocyte damage, and the complete absence of melanocytes in affected regions of the skin. Yee et al. and colleagues provided, for the first time, direct evidence that antigen-specific immunotherapy can target not only antigen-positive tumor cells in vivo but also normal tissues expressing the shared tumor antigen.

**Discussion**

Currently there are no US FDA approved ACT therapies. Although within the last decade, there has been an explosion of knowledge in genetics, biochemistry, immunology, and oncology. The new approaches being made will revolutionize cancer therapy as did the advent of transfusion medicine.
Adoptive transfer therapy with TILs requires the isolation of T cells from fresh patient biopsy specimens, and the progressive selection of tumor-specific T cells *ex vivo* using high levels of IL-2 and various cell culture approaches. The adoptive transfer of these cells showed promise in preclinical models,\(^\text{31}\) but clinical experiences, with perhaps one exception,\(^\text{32}\) were almost uniformly disappointing. However, recent studies at the National Cancer Institute suggest that prior host conditioning with chemotherapy increases the response to adoptive immunotherapy with TILs. Unfortunately, in the absence of a randomized clinical trial, it is not possible to determine how much lymphoablative chemotherapy, high-dose IL-2 administration, and TIL therapy contributed to the promising results in these recent trials conducted by Dudley et al. and colleagues, 2005.

Technical issues with the production of tumor-specific T cells currently present a formidable barrier to conducting randomized clinical trials using TILs. Only 30%–40% of biopsy specimens yield satisfactory T cell populations, and the process is labor, cost, and time intensive, requiring about 6 weeks to produce the T cells for infusion. These obstacles were reported by Dudley et al. and colleagues, 2003. Therefore, randomized trials based on a rigorous intent-to-treat analysis (in which all data from all patients are included in the data analysis and any patients who are discontinued or otherwise non-evaluable are considered to be treatment failures) cannot be performed using currently available tissue culture technologies. The trials reported to date, and in this review, have been performed based on an ad hoc, as-treated analysis plan. It is also important to mention, nearly all clinical trials with TILs have involved patients with MMM because of the surgical availability of tumor biopsy tissue. However, when the limitations of current tissue culture technology are overcome, this therapeutic approach shows great promise in impacting the survival of those afflicted with epithelial cancers.
To answer the clinical question: Is there a benefit to treatment with ACT following lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma? Following review of the outcomes generated from the four clinical trials, this question remains difficult to answer. The ability to have the continuity of researchers involved, and relatively the same methods, made this topic interesting to examine. The future of this particular form of treatment looks very promising, but currently the response statistics are low. The patients must take into account quality of life, whether to spend his or her last days as chosen, or dedicate a substantial amount of time to treatment in a clinical atmosphere which may or may not prolong his or her life.

**Conclusion**

In a time where restrictions on stem-cell research are being lifted, the hope for cure and improved treatment for those suffering from chronic and terminal illness continues to grow. The capacity to study stem cells will certainly allow insight into our ability to manipulate cellular activity, growth, and death.

The goal of this review was to educate the reader about the clinical trials of ACT for the treatment of MMM. This knowledge is based on lymphocyte and cancer biology, which has only begun to crest the horizon in recent years. Nevertheless, lessons can be learned from previous trials which failed to achieve their clinical goal. Like all of medicine, there has always been a process of trial and error, proof and cure. Given time to overcome technological and logistical obstacles, immunotherapy will be at the forefront of successful cancer treatment in the very near future.
**TABLE I:** The ABCD Checklist for Detecting Cutaneous Melanoma

<table>
<thead>
<tr>
<th>Checklist item</th>
<th>Reassuring elements</th>
<th>Non-reassuring elements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> = Asymmetry</td>
<td>Symmetric (benign)</td>
<td>Asymmetric (malignant melanoma)</td>
</tr>
<tr>
<td>Suggestive of melanoma if the lesion is bisected and the halves are not identical</td>
<td>Borders are even (benign)</td>
<td>Borders are irregular (malignant melanoma)</td>
</tr>
<tr>
<td><strong>B</strong> = Border irregularity</td>
<td>One shade/even color (benign)</td>
<td>Two or more shades/uneven color (malignant melanoma)</td>
</tr>
<tr>
<td>Suggestive of melanoma if the border is uneven or ragged</td>
<td>Diameter &lt;6 mm (benign)</td>
<td>Diameter &gt;6mm (malignant melanoma)</td>
</tr>
<tr>
<td><strong>C</strong> = Color variation</td>
<td>Diameter</td>
<td></td>
</tr>
<tr>
<td>Suggestive of melanoma if there is more than one shade of pigment</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D</strong> = Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suggestive of melanoma if the diameter is greater than 6 mm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Biopsy should be strongly considered if one or more non-reassuring elements are present.

### TABLE II: Seven-Point Checklist for Suspected Malignant Melanoma

| Major signs* | | Minor signs** |
|--------------|________________|________________|
| Change in size | | Inflammation |
| Change in shape | | Crusting or bleeding |
| Change in color | | Sensory change |
| Diameter of 7 mm or more | | |

*One or more major signs: refer for expeditious biopsy; additional presence of one or more minor signs increases the possibility of melanoma.

**Three or four minor signs without a major sign: consider referral.

Information from Whited JD, Grichnik JM. Does this patient have a mole or a melanoma? JAMA 1998; 279:696-701.

### TABLE III: Breslow's Microstages and Clark's Levels for Melanoma

<table>
<thead>
<tr>
<th>Breslow's microstages</th>
<th>Five-year survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1: &lt;0.76 mm</td>
<td>&gt;98</td>
</tr>
<tr>
<td>Stage 2: 0.76 to 1.49 mm</td>
<td>87 to 94</td>
</tr>
<tr>
<td>Stage 3: 1.50 to 3.99 mm</td>
<td>66 to 83</td>
</tr>
<tr>
<td>Stage 4: &gt;4.0 mm</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clark's levels</th>
<th>Five-year survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level I: Intraepidermal</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Level II: Extends into the papillary dermis</td>
<td>95</td>
</tr>
<tr>
<td>Level III: Fills the papillary dermis</td>
<td>82</td>
</tr>
<tr>
<td>Level IV: Extends into the reticular dermis</td>
<td>71</td>
</tr>
<tr>
<td>Level V: Invades the subcutaneous tissue</td>
<td>49</td>
</tr>
</tbody>
</table>

Figure I: Distribution of melanoma by body site and sex.

References

1. ANTHONY F. JERANT, M.D., University of California, Davis, School of Medicine, Sacramento, California JENNIFER T. JOHNSON, M.D., Eisenhower Army Medical Center, Fort Gordon, Georgia CATHERINE DEMASTES SHERIDAN, M.D., Buedingen Army Health Clinic, Buedingen, Germany TIMOTHY J. CAFFREY, M.D., Commander, Vicenza Army Health Clinic, Vicenza, Italy: Early Detection and Treatment of Skin Cancer. Published by the American Academy of Family Physicians; July 15, 2000.


12. CDKN2A cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4) from Entrez Gene


15. Bene, NI, et al. Mohs micrographic surgery is accurate 95.1% of the time for melanoma in situ: a prospective study of 167 cases Dermatol Surg. 2008 May;34(5):660-4. Cure rate as high as 98% for small melanoma in situ, and as high as 95% noted for lentigo maligna variant of melanoma in situ has been reported with Mohs’ surgery.


Articles Reviewed:

A Phase I Study of Nonmyeloablative Chemotherapy and Adoptive Transfer of Autologous Tumor Antigen-Specific T Lymphocytes in Patients With Metastatic Melanoma


*Surgery Branch, National Cancer Institute, and †Department of Transfusion Medicine, National Institutes of Health, Bethesda, Maryland, U.S.A.

Adoptive Cell Transfer Therapy Following Non-Myeloablative but Lymphodepleting Chemotherapy for the Treatment of Patients With Refractory Metastatic Melanoma

Journal of Clinical Oncology, Volume 23 - Number 10 - April 1, 2005

Adoptive Cell Therapy for Patients with Melanoma, Using Tumor-Infiltrating Lymphocytes Genetically Engineered to Secrete Interleukin-2
BIANCA HEEMSKERK1, KE LIU1,2, MARK. E. DUDLEY1, LAURA A. JOHNSON1, ANDREW KAISER1, STEPHANIE DOWNEY1, ZHILI ZHENG1, THOMAS E. SHELTON1, KANT MATSUDA3, PAUL F. ROBBINS1, RICHARD A. MORGAN1, and STEVEN A. ROSENBERG1
1Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
2U.S. Food and Drug Administration, Rockville, MD 20852.
3Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.


Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes

Surgery Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 10 Center Drive, Bethesda, MD 20892, USA.


Melanocyte Destruction after Antigen-specific Immunotherapy of Melanoma: Direct Evidence of T Cell–mediated Vitiligo
By Cassian Yee,* ‡ John A. Thompson, ‡ Patrick Roche, I David R. Byrd, § Peter P. Lee, ¶ Michael Piepkorn, ‡ Karla Kenyon,* Mark M. Davis, ¶ Stanley R. Riddell, *‡ and Philip D. Greenberg
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Adoptive T cell therapy for cancer in the clinic by Carl H. June
Abramson Family Cancer Research Institute and Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

The Journal of Clinical Investigation http://www.jci.org Volume 117 - Number 6 - June 200